

PAT/2927P  
Case 2927

META-SUBSTITUTED PHENYLENE SULPHONAMIDE DERIVATIVES

Field of the Invention

5 The present invention relates to pharmaceutical  
agents (compounds) which are useful as  $\alpha_v\beta_3$  integrin  
antagonists or inhibitors and as such are useful in  
pharmaceutical compositions and in methods for treating  
conditions mediated by  $\alpha_v\beta_3$  by inhibiting or  
antagonizing  $\alpha_v\beta_3$  integrins.

10

Background of the Invention

Integrins are a group of cell surface  
glycoproteins which mediate cell adhesion and therefore  
are useful mediators of cell adhesion interactions  
15 which occur during various biological processes.  
Integrins are heterodimers composed of noncovalently  
linked  $\alpha$  and  $\beta$  polypeptide subunits. Currently eleven  
different  $\alpha$  subunits have been identified and six  
different  $\beta$  subunits have been identified. The various  
20  $\alpha$  subunits can combine with various  $\beta$  subunits to form  
distinct integrins.

The integrin identified as  $\alpha_v\beta_3$  (also known as the  
vitronectin receptor) has been identified as an  
integrin which plays a role in various conditions or  
25 disease states including tumor metastasis, solid tumor  
growth (neoplasia), osteoporosis, Paget's disease,  
humoral hypercalcemia of malignancy, angiogenesis,  
including tumor angiogenesis, retinopathy, arthritis,  
including rheumatoid arthritis, periodontal disease,  
30 psoriasis and smooth muscle cell migration (e.g.  
restenosis). Additionally, it has been found that such  
agents would be useful as antivirals, antifungals and  
antimicrobials. Thus, compounds which selectively  
inhibit or antagonize  $\alpha_v\beta_3$  would be beneficial for  
35 treating such conditions.

It has been shown that the  $\alpha_v\beta_3$  integrin and other  $\alpha_v$  containing integrins bind to a number of Arg-Gly-Asp (RGD) containing matrix macromolecules. Compounds containing the RGD sequence mimic extracellular matrix ligands so as to bind to cell surface receptors. However, it is also known that RGD peptides in general are non-selective for RGD dependent integrins. For example, most RGD peptides which bind to  $\alpha_v\beta_3$  also bind to  $\alpha_v\beta_5$ ,  $\alpha_v\beta_1$  and  $\alpha_{IIb}\beta_3$ . Antagonism of platelet  $\alpha_{IIb}\beta_3$  (also known as the fibrinogen receptor) is known to block platelet aggregation in humans. In order to avoid bleeding side-effects when treating the conditions or disease states associated with the integrin  $\alpha_v\beta_3$ , it would be beneficial to develop compounds which are selective antagonists of  $\alpha_v\beta_3$  as opposed to  $\alpha_{IIb}\beta_3$ .

Tumor cell invasion occurs by a three step process: 1) tumor cell attachment to extracellular matrix; 2) proteolytic dissolution of the matrix; and 3) movement of the cells through the dissolved barrier. This process can occur repeatedly and can result in metastases at sites distant from the original tumor.

Seftor et al. (Proc. Natl. Acad. Sci. USA, Vol. 89 (1992) 1557-1561) have shown that the  $\alpha_v\beta_3$  integrin has a biological function in melanoma cell invasion. Montgomery et al., (Proc. Natl. Acad. Sci. USA, Vol. 91 (1994) 8856-60) have demonstrated that the integrin  $\alpha_v\beta_3$  expressed on human melanoma cells promotes a survival signal, protecting the cells from apoptosis. Mediation of the tumor cell metastatic pathway by interference with the  $\alpha_v\beta_3$  integrin cell adhesion receptor to impede tumor metastasis would be beneficial.

Brooks et al. (Cell, Vol. 79 (1994) 1157-1164) have demonstrated that antagonists of  $\alpha_v\beta_3$  provide a therapeutic approach for the treatment of neoplasia (inhibition of solid tumor growth) since systemic administration of  $\alpha_v\beta_3$  antagonists causes dramatic

regression of various histologically distinct human tumors.

5 The adhesion receptor integrin  $\alpha_v\beta_3$  was identified as a marker of angiogenic blood vessels in chick and man and therefore such receptor plays a critical role in angiogenesis or neovascularization. Angiogenesis is characterized by the invasion, migration and proliferation of smooth muscle and endothelial cells. Antagonists of  $\alpha_v\beta_3$  inhibit this process by selectively  
10 promoting apoptosis of cells in neovasculature. The growth of new blood vessels, or angiogenesis, also contributes to pathological conditions such as diabetic retinopathy (Adonis et al., Amer. J. Ophthal., Vol. 118, (1994) 445-450) and rheumatoid arthritis (Peacock  
15 et al., J. Exp. Med., Vol. 175, (1992), 1135-1138). Therefore,  $\alpha_v\beta_3$  antagonists would be useful therapeutic targets for treating such conditions associated with neovascularization (Brooks et al., Science, Vol. 264, (1994), 569-571).

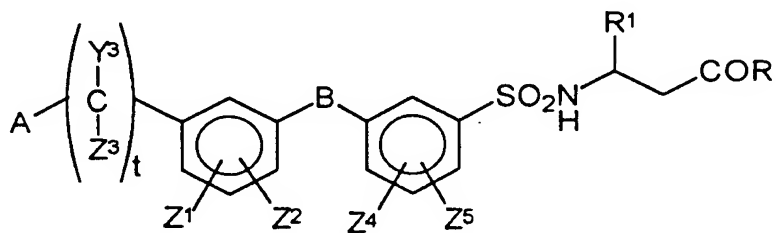
20 It has been reported that the cell surface receptor  $\alpha_v\beta_3$  is the major integrin on osteoclasts responsible for attachment to bone. Osteoclasts cause bone resorption and when such bone resorbing activity exceeds bone forming activity it results in  
25 osteoporosis (a loss of bone), which leads to an increased number of bone fractures, incapacitation and increased mortality. Antagonists of  $\alpha_v\beta_3$  have been shown to be potent inhibitors of osteoclastic activity both *in vitro* [Sato et al., J. Cell. Biol., Vol. 111  
30 (1990) 1713-1723] and *in vivo* [Fisher et al., Endocrinology, Vol. 132 (1993) 1411-1413]. Antagonism of  $\alpha_v\beta_3$  leads to decreased bone resorption and therefore restores a normal balance of bone forming and resorbing activity. Thus it would be beneficial to provide  
35 antagonists of osteoclast  $\alpha_v\beta_3$  which are effective inhibitors of bone resorption and therefore are useful in the treatment or prevention of osteoporosis.

The role of the  $\alpha_v\beta_3$  integrin in smooth muscle cell migration also makes it a therapeutic target for prevention or inhibition of neointimal hyperplasia which is a leading cause of restenosis after vascular procedures (Choi et al., J. Vasc. Surg. Vol. 19(1) (1994) 125-34). Prevention or inhibition of neointimal hyperplasia by pharmaceutical agents to prevent or inhibit restenosis would be beneficial.

White (Current Biology, Vol. 3(9)(1993) 596-599) has reported that adenovirus uses  $\alpha_v\beta_3$  for entering host cells. The integrin appears to be required for endocytosis of the virus particle and may be required for penetration of the viral genome into the host cell cytoplasm. Thus compounds which inhibit  $\alpha_v\beta_3$  would find usefulness as antiviral agents.

#### Summary of the Invention

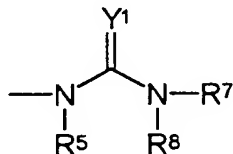
The present invention relates to a class of compounds represented by the Formula I



or a pharmaceutically acceptable salt thereof, wherein

B is selected from the group consisting of  $-\text{CONR}^{50}-$  and  $-\text{SO}_2\text{NR}^{50}-$ ;

A is



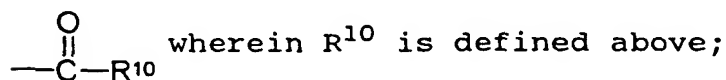
wherein  $Y^1$  is selected from the group consisting of  $N-R^2$ , O, and S;

$R^2$  is selected from the group consisting of H;  
5 alkyl; aryl; hydroxy; alkoxy; cyano; nitro; amino;  
alkenyl; alkynyl; alkyl optionally substituted  
with one or more substituent selected from lower  
alkyl, halogen, hydroxyl, haloalkyl, cyano, nitro,  
10 carboxyl, amino, alkoxy, aryl or aryl optionally  
substituted with one or more halogen, haloalkyl,  
lower alkyl, alkoxy, cyano, alkylsulfonyl,  
alkylthio, nitro, carboxyl, amino, hydroxyl,  
sulfonic acid, sulfonamide, aryl, fused aryl,  
15 monocyclic heterocycles, or fused monocyclic  
heterocycles; aryl optionally substituted with one  
or more substituent selected from halogen,  
haloalkyl, hydroxy, lower alkyl, alkoxy,  
methylenedioxy, ethylenedioxy, cyano, nitro,  
20 alkylthio, alkylsulfonyl, sulfonic acid,  
sulfonamide, carboxyl derivatives, amino, aryl,  
fused aryl, monocyclic heterocycles and fused  
monocyclic heterocycle; monocyclic heterocycles;  
and monocyclic heterocycles optionally substituted  
25 with one or more substituent selected from  
halogen, haloalkyl, lower alkyl, alkoxy, amino,  
nitro, hydroxy, carboxyl derivatives, cyano,  
alkylthio, alkylsulfonyl, sulfonic acid,  
sulfonamide, aryl or fused aryl; or  
30  $R^2$  taken together with  $R^7$  forms a 4-12 membered  
dinitrogen containing heterocycle optionally  
substituted with one or more substituent selected  
from the group consisting of lower alkyl, hydroxy  
and phenyl;  
35 or  $R^2$  taken together with  $R^7$  forms a 5 membered  
heteroaromatic ring;

or  $R^2$  taken together with  $R^7$  forms a 5 membered heteroaromatic ring fused with a phenyl group;

5  $R^7$  (when not taken together with  $R^2$ ) and  $R^8$  are independently selected from the group consisting of H; alkyl; alkenyl; alkynyl; aralkyl; cycloalkyl; bicycloalkyl; aryl; acyl; benzoyl; alkyl optionally substituted with one or more  
10 substituent selected from lower alkyl, halogen, hydroxy, haloalkyl, cyano, nitro, carboxyl derivatives, amino, alkoxy, thio, alkylthio, sulfonyl, aryl, aralkyl, aryl optionally substituted with one or more substituent selected from halogen, haloalkyl, lower alkyl, alkoxy,  
15 methylenedioxy, ethylenedioxy, alkylthio, haloalkylthio, thio, hydroxy, cyano, nitro, carboxyl derivatives, aryloxy, amido, acylamino, amino, alkylamino, dialkylamino, trifluoroalkoxy, trifluoromethyl, sulfonyl, alkylsulfonyl,  
20 haloalkylsulfonyl, sulfonic acid, sulfonamide, aryl, fused aryl, monocyclic heterocycles, fused monocyclic heterocycles; aryl optionally substituted with one or more substituent selected from halogen, haloalkyl, lower alkyl, alkoxy,  
25 methylenedioxy, ethylenedioxy, alkylthio, haloalkylthio, thio, hydroxy, cyano, nitro, carboxyl derivatives, aryloxy, amido, acylamino, amino, alkylamino, dialkylamino, trifluoroalkoxy, trifluoromethylsulfonyl, alkylsulfonyl, sulfonic  
30 acid, sulfonamide, aryl, fused aryl, monocyclic heterocycles, or fused monocyclic heterocycles; monocyclic heterocycles; monocyclic heterocycles optionally substituted with one or more substituent selected from halogen, haloalkyl,  
35 lower alkyl, alkoxy, aryloxy, amino, nitro, hydroxy, carboxyl derivatives, cyano, alkylthio, alkylsulfonyl, aryl, fused aryl; monocyclic and

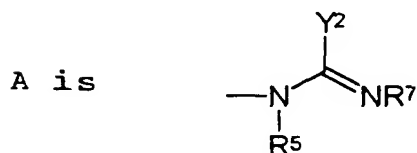
bicyclic heterocyclicalkyls;  $-\text{SO}_2\text{R}^{10}$  wherein  $\text{R}^{10}$  is selected from the group consisting of alkyl, aryl and monocyclic heterocycles, all optionally substituted with one or more substituent selected from the group consisting of halogen, haloalkyl, alkyl, alkoxy, cyano, nitro, amino, acylamino, trifluoroalkyl, amido, alkylaminosulfonyl, alkylsulfonyl, alkylsulfonylamino, alkylamino, dialkylamino, trifluoromethylthio, trifluoroalkoxy, trifluoromethylsulfonyl, aryl, aryloxy, thio, alkylthio, and monocyclic heterocycles; and



or  $\text{NR}^7$  and  $\text{R}^8$  taken together form a 4-12 membered mononitrogen containing monocyclic or bicyclic ring optionally substituted with one or more substituent selected from lower alkyl, carboxyl derivatives, aryl or hydroxy and wherein said ring optionally contains a heteroatom selected from the group consisting of O, N and S;

$\text{R}^5$  is selected from the group consisting of H, alkyl, alkenyl, alkynyl, benzyl, and phenethyl;

or



15 of the free acid, all pharmaceutic  
salts thereof; and

$Y^3$  and  $Z^3$  are independently selected  
consisting of H, alkyl, aryl, cycloalkyl.  
20

It is another object of the invention  
pharmaceutical compositions comprising consisting of H,  
Formula I. Such compounds and compositions consisting of H,  
25 in selectively inhibiting or antagonizing integrin and therefore in another embodiment, haloalkyl,  
present invention relates to a method of, amino,  
inhibiting or antagonizing the  $\alpha_v\beta_3$  integrin and cyano and nitro;  
invention further involves treating or  
30 pathological conditions associated therewith:  
osteoporosis, humoral hypercalcemia of  
Paget's disease, tumor metastasis, stroke, the group  
(neoplasia), angiogenesis, including tumor, in  $R^3$  and  $R^4$  are  
angiogenesis, retinopathy including diabetic retinopathy consisting  
35 retinopathy, arthritis, including rheumatoid arthritis; haloalkyl;  
periodontal disease, psoriasis, smooth muscle and in the case  
migration and restenosis in a mammal in a pharmaceutically acceptable

selected from the group  
haloalkyl and

invention to provide  
compounds of the  
inventions are useful  
inhibiting the  $\alpha_v\beta_3$

- 9 -

$R^1$  is selected from the group consisting of the  
alkyl, alkenyl, alkynyl, aryl and cycloalkyl and is  
substituted with one or more substituents. The  
from the group consisting of haloalkyl, hydroxy,  
5 hydroxy, alkoxy, aryloxy, aralkoxy, aminoalkyl,  
aminoalkyl, carboxyl derivatives, malonic acid, tumor growth  
inhibitors, and tumor growth inhibitors;  
n is an integer 0, 1 or 2; tumor growth inhibitors;  
aromatic  
10  $R$  is  $X-R^3$  wherein  $X$  is selected from the group consisting of  
maternal arthritis, consisting of O, S and  $NR^4$ , where  $R^4$  is  
independently selected from the group consisting of hydrogen,  
alkyl, alkenyl, alkynyl, aryl, cycloalkyl, and heterocycloalkyl;



treatment. Additionally, such pharmaceutical agents are useful as antiviral agents, and antimicrobials.

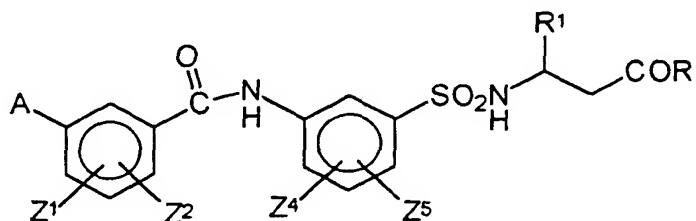
### Detailed Description

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The present invention relates to a class of compounds represented by the Formula I, described above.

10 A preferred embodiment of the present invention is a compound of the Formula II

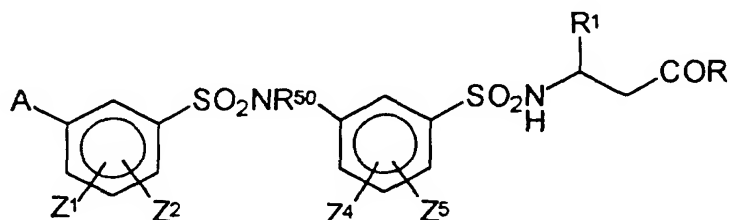
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Another preferred embodiment of the present invention is a compound of the Formula III

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The invention further relates to pharmaceutical compositions containing therapeutically effective amounts of the compounds of Formulas I-III.

35 The invention also relates to a method of selectively inhibiting or antagonizing the  $\alpha_v\beta_3$  integrin and more specifically relates to a method of inhibiting bone resorption, periodontal disease, osteoporosis,

humoral hypercalcemia of malignancy, Paget's disease, tumor metastasis, solid tumor growth (neoplasia), angiogenesis, including tumor angiogenesis, retinopathy including diabetic retinopathy, arthritis, including  
5 rheumatoid arthritis, smooth muscle cell migration and restenosis by administering a therapeutically effective amount of a compound of the Formula I-III to achieve such inhibition together with a pharmaceutically acceptable carrier.

10 The following is a list of definitions of various terms used herein:

As used herein, the terms "alkyl" or "lower alkyl" refer to a straight chain or branched chain hydrocarbon radicals having from about 1 to about 10 carbon atoms, and more preferably 1 to about 6 carbon atoms.

5 Examples of such alkyl radicals are methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, t-butyl, pentyl, neopentyl, hexyl, isohexyl, and the like.

As used herein the terms "alkenyl" or "lower alkenyl" refer to unsaturated acyclic hydrocarbon radicals containing at least one double bond and 2 to about 6 carbon atoms, which carbon-carbon double bond may have either cis or trans geometry within the alkenyl moiety, relative to groups substituted on the  
10 double bond carbons. Examples of such groups are  
15 ethenyl, propenyl, butenyl, isobutenyl, pentenyl, hexenyl and the like.

As used herein the terms "alkynyl" or "lower alkynyl" refer to acyclic hydrocarbon radicals  
20 containing one or more triple bonds and 2 to about 6 carbon atoms. Examples of such groups are ethynyl, propynyl, butynyl, pentynyl, hexynyl and the like.

The term "cycloalkyl" as used herein means saturated or partially unsaturated cyclic carbon  
25 radicals containing 3 to about 8 carbon atoms and more preferably 4 to about 6 carbon atoms. Examples of such

cycloalkyl radicals include cyclopropyl, cyclopropenyl, cyclobutyl, cyclopentyl, cyclohexyl, 2-cyclohexen-1-yl, and the like.

5 The term "aryl" as used herein denotes aromatic ring systems composed of one or more aromatic rings. Preferred aryl groups are those consisting of one, two or three aromatic rings. The term embraces aromatic radicals such as phenyl, pyridyl, naphthyl, thiophene, furan, biphenyl and the like.

10 As used herein, the term "cyano" is represented by a radical of the formula  $\text{---}\text{CN}$ .

The terms "hydroxy" and "hydroxyl" as used herein are synonymous and are represented by a radical of the formula  $\text{---}\text{OH}$ .

15 The term "lower alkylene" or "alkylene" as used herein refers to divalent linear or branched saturated hydrocarbon radicals of 1 to about 6 carbon atoms.

As used herein the term "alkynylene" or "lower alkynylene" refers to an alkylene radical wherein at  
20 least one bond between the carbon atoms is unsaturated and such unsaturation forms a triple bond.

As used herein the term "alkenylene" or "lower alkenylene" refers to an alkylene radical wherein at  
25 least one bond between the carbon atoms is unsaturated and such unsaturation produces a double bond in cis or transconformation.

As used herein the term "alkoxy" refers to straight or branched chain oxy containing radicals of the formula  $\text{---OR}^{20}$ , wherein  $\text{R}^{20}$  is an alkyl group as  
30 defined above. Examples of alkoxy groups encompassed include methoxy, ethoxy, n-propoxy, n-butoxy, isopropoxy, isobutoxy, sec-butoxy, t-butoxy and the like.

As used herein the terms "arylalkyl" or "aralkyl" refer to a radical of the formula  $\text{---R}^{22}\text{---R}^{21}$  wherein  $\text{R}^{21}$  is aryl as defined above and  $\text{R}^{22}$  is an alkylene as defined above. Examples of aralkyl groups include benzyl, pyridylmethyl, naphthylpropyl, phenethyl and the like.

As used herein the term "aralkoxy" or "arylakoxy" refers to a radical of the formula  $\text{---OR}^{53}$  wherein  $\text{R}^{53}$  is aralkyl as defined above.

As used herein the term "nitro" is represented by a radical of the formula  $\text{---NO}_2$ .

As used herein the term "halo" or "halogen" refers to bromo, chloro, fluoro or iodo.

As used herein the term "haloalkyl" refers to alkyl groups as defined above substituted with one or more of the same or different halo groups at one or more carbon atom. Examples of haloalkyl groups include trifluoromethyl, dichloroethyl, fluoropropyl and the like.

As used herein the term "carboxyl" or "carboxy" refers to a radical of the formula  $\text{---COOH}$ .

As used herein the term "aminoalkyl" refers to a radical of the formula  $\text{---R}^{54}\text{---NH}_2$  wherein  $\text{R}^{54}$  is lower alkylene as defined above.

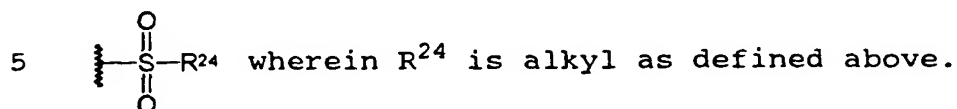
As used herein the term "carboxyl derivative"

refers to a radical of the formula  $\text{---}\overset{\text{Y}^6}{\underset{\text{||}}{\text{C}}}\text{---Y}^7\text{R}^{23}$  wherein

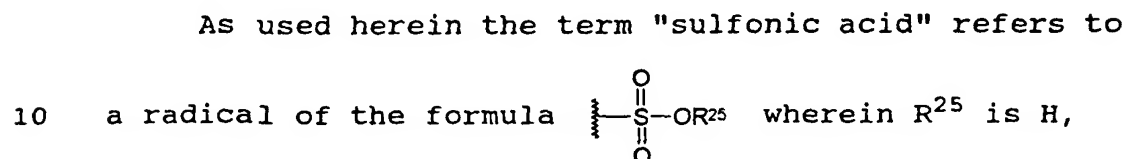
$\text{Y}^6$  and  $\text{Y}^7$  are independently selected from the group consisting of O, N or S and  $\text{R}^{23}$  is selected from the group consisting of H, alkyl, aralkyl or aryl as defined above.

As used herein the term "amino" is represented by a radical of the formula  $\text{-NH}_2$ .

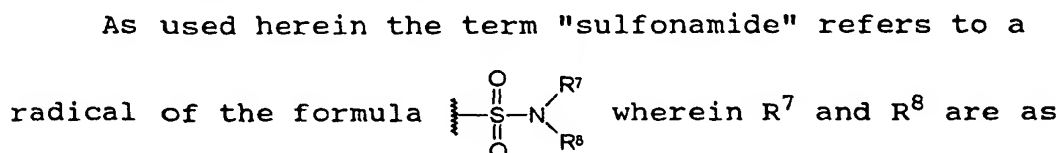
As used herein the term "alkylsulfonyl" or "alkylsulfone" refers to a radical of the formula



As used herein the term "alkylthio" refers to a radical of the formula  $\text{-SR}^{24}$  wherein  $\text{R}^{24}$  is alkyl as defined above.



alkyl or aryl as defined above.



defined above.

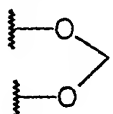
15     As used herein the term "fused aryl" refers to an aromatic ring such as the aryl groups defined above fused to one or more phenyl rings. Embraced by the term "fused aryl" is the radical naphthyl.

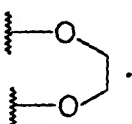
20     As used herein the terms "monocyclic heterocycle" or "monocyclic heterocyclic" refer to a monocyclic ring containing from 4 to about 12 atoms, and more preferably from 5 to about 10 atoms, wherein 1 to 3 of the atoms are heteroatoms selected from the group consisting of oxygen, nitrogen and sulfur with the  
25     understanding that if two or more different heteroatoms are present at least one of the heteroatoms must be nitrogen. Representative of such monocyclic

heterocycles are imidazole, furan, pyridine, oxazole, pyran, triazole, thiophene, pyrazole, thiazole, thiadiazole, and the like.

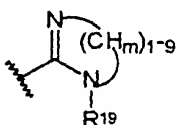
As used herein the term "fused monocyclic heterocycle" refers to a monocyclic heterocycle as defined above with a benzene fused thereto. Examples of such fused monocyclic heterocycles include benzofuran, benzopyran, benzodioxole, benzothiazole, benzothiophene, benzimidazole and the like.

As used herein the term "methylenedioxy" refers to

the radical  and the term "ethylenedioxy" refers

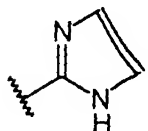
to the radical .

As used herein the term "4-12 membered dinitrogen containing heterocycle" refers to a radical of the

formula  wherein m is 1 or 2 and R<sup>19</sup> is

H, alkyl, aryl, or aralkyl and more preferably refers to 4-9 membered ring and includes rings such as imidazoline.

As used herein the term "5-membered heteroaromatic ring" includes for example a radical of the formula

 and "5-membered heteroaromatic ring fused

with a phenyl" refers to such a "5-membered

heteroaromatic ring" with a phenyl fused thereto. Representative of such 5-membered heteroaromatic rings fused with a phenyl is benzimidazole.

As used herein the term "bicycloalkyl" refers to a bicyclic hydrocarbon radical containing 6 to about 12 carbon atoms which is saturated or partially unsaturated.

As used herein the term "acyl" refers to a radical of the formula  $\begin{array}{c} \text{O} \\ \parallel \\ \text{---C---R}^{26} \end{array}$  wherein  $\text{R}^{26}$  is alkyl, alkenyl,

alkynyl, aryl or aralkyl as defined above. Encompassed by such radical are the groups acetyl, benzoyl and the like.

As used herein the term "thio" refers to a radical of the formula  $\begin{array}{c} \text{---SH} \\ | \\ | \\ | \end{array}$ .

As used herein the term "sulfonyl" refers to a

radical of the formula  $\begin{array}{c} \text{O} \\ \parallel \\ \text{---S---R}^{27} \\ \parallel \\ \text{O} \end{array}$  wherein  $\text{R}^{27}$  is alkyl,

aryl or aralkyl as defined above.

As used herein the term "haloalkylthio" refers to a radical of the formula  $\text{---S---R}^{28}$  wherein  $\text{R}^{28}$  is haloalkyl as defined above.

As used herein the term "aryloxy" refers to a radical of the formula  $\begin{array}{c} \text{---OR}^{29} \\ | \\ | \\ | \end{array}$  wherein  $\text{R}^{29}$  is aryl as defined above.

As used herein the term "acylamino" refers to a radical of the formula 
$$\text{R}^{30}-\overset{\text{O}}{\parallel}{\text{C}}-\text{NH}-$$
 wherein  $\text{R}^{30}$  is alkyl,

aralkyl or aryl as defined above.

As used herein the term "amido" refers to a radical of the formula 
$$\text{---R}^{31}-\overset{\text{O}}{\parallel}{\text{C}}-\text{NH}_2$$
 wherein  $\text{R}^{31}$  is a bond

or alkylene as defined above.

As used herein the term "alkylamino" refers to a radical of the formula  $-\text{NHR}^{32}$  wherein  $\text{R}^{32}$  is alkyl as defined above.

As used herein the term "dialkylamino" refers to a radical of the formula  $-\text{NR}^{33}\text{R}^{34}$  wherein  $\text{R}^{33}$  and  $\text{R}^{34}$  are the same or different alkyl groups as defined above.

As used herein the term "trifluoromethyl" refers to a radical of the formula  $- \text{CF}_3$ .

As used herein the term "trifluoroalkoxy" refers to a radical of the formula  $\text{F}_3\text{C}-\text{R}^{35}-\text{O}-$  wherein  $\text{R}^{35}$  is a bond or an alkylene as defined above.

As used herein the term "alkylaminosulfonyl" refers to a radical of the formula 
$$\text{R}^{36}-\underset{\text{H}}{\text{N}}-\overset{\text{O}}{\parallel}{\text{S}}-$$
 wherein

$\text{R}^{36}$  is alkyl as defined above.



As used herein the term "alkylsulfonylamino"

refers to a radical of the formula 
$$R^{36}-\overset{\overset{O}{\parallel}}{\underset{\underset{O}{\parallel}}{S}}-NH-\text{---}$$

wherein  $R^{36}$  is alkyl as defined above.

As used herein the term "trifluoromethylthio"

5 refers to a radical of the formula  $F_3C-S-\text{---}$ .

As used herein the term "trifluoromethylsulfonyl"

refers to a radical of the formula 
$$F_3C-\overset{\overset{O}{\parallel}}{\underset{\underset{O}{\parallel}}{S}}-\text{---}$$
.

As used herein the term "4-12 membered mono-nitrogen containing monocyclic or bicyclic ring" refers  
10 to a saturated or partially unsaturated monocyclic or bicyclic ring of 4-12 atoms and more preferably a ring of 4-9 atoms wherein one atom is nitrogen. Such rings may optionally contain additional heteroatoms selected from nitrogen, oxygen or sulfur. Included within this  
15 group are morpholine, piperidine, piperazine, thiomorpholine, pyrrolidine, proline, azacycloheptene and the like.

As used herein the term "benzyl" refers to the

radical  $\text{---CH}_2-\text{C}_6\text{H}_5$ .

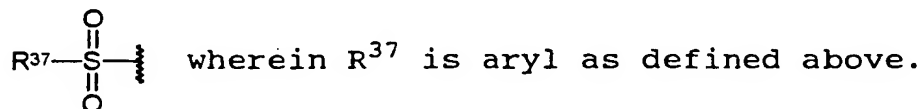
20 As used herein the term "phenethyl" refers to the

radical  $\text{---CH}_2\text{CH}_2-\text{C}_6\text{H}_5$ .

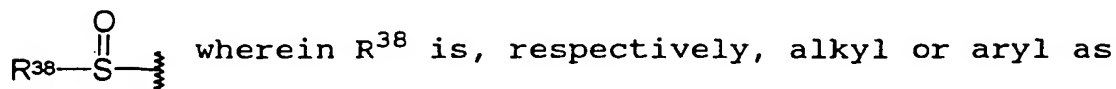
As used herein the term "4-12 membered mono-nitrogen containing sulfur or oxygen containing heterocyclic ring" refers to a ring consisting of 4 to  
25 12 atoms and more preferably 4 to 9 atoms wherein at

least one atom is a nitrogen and at least one atom is oxygen or sulfur. Encompassed within this definition are rings such as thiazoline and the like.

As used herein the term "arylsulfonyl" or  
5 "arylsulfone" refers to a radical of the formula



As used herein the terms "alkylsulfoxide" or "arylsulfoxide" refer to radicals of the formula



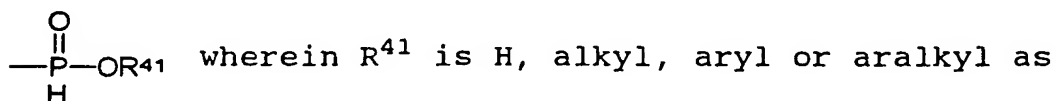
10 defined above.

As used herein the term "phosphonic acid

derivative" refers to a radical of the formula  $\text{P}(=\text{O})(\text{OR}^{39})(\text{OR}^{40})-$

wherein  $\text{R}^{39}$  and  $\text{R}^{40}$  are the same or different H, alkyl, aryl or aralkyl.

15 As used herein the term "phosphinic acid derivatives" refers to a radical of the formula



defined above.

As used herein the term "arylthio" refers to a  
20 radical of the formula  $- \text{SR}^{42}$  wherein  $\text{R}^{42}$  is aryl as  
defined above.

As used herein the term "monocyclic heterocycle thio" refers to a radical of the formula  $\text{---SR}^{43}$

wherein  $\text{R}^{43}$  is a monocyclic heterocycle radical as defined above.

5 As used herein the terms "monocyclic heterocycle sulfoxide" and "monocyclic heterocycle sulfone" refer,

respectively, to radicals of the formula  $\text{---S(=O)R}^{43}$  and

$\text{---S(=O)}_2\text{R}^{43}$  wherein  $\text{R}^{43}$  is a monocyclic heterocycle

radical as defined above.

10 The term "composition" as used herein means a product which results from the mixing or combining of more than one element or ingredient.

The term "pharmaceutically acceptable carrier", as used herein means a pharmaceutically-acceptable  
15 material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a chemical agent.

The term "therapeutically effective amount" shall  
20 mean that amount of drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system or animal that is being sought by a researcher or clinician.

The following is a list of abbreviations and the  
25 corresponding meanings as used interchangeably herein:

$^1\text{H-NMR}$  = proton nuclear magnetic resonance  
AcOH = acetic acid  
BH<sub>3</sub>-THF = borane-tetrahydrofuran complex  
30 BOC = tert-butoxycarbonyl

Cat. = catalytic amount  
CH<sub>2</sub>Cl<sub>2</sub> = dichloromethane  
CH<sub>3</sub>CN = acetonitrile  
CH<sub>3</sub>I = iodomethane  
5 CHN analysis = carbon/hydrogen/nitrogen elemental  
analysis  
CHNCl analysis = carbon/hydrogen/nitrogen/chlorine  
elemental analysis  
CHNS analysis = carbon/hydrogen/nitrogen/sulfur  
10 elemental analysis  
DCC = 1,3-dicyclohexylcarbodiimide  
DIEA = diisopropylethylamine  
DMA = N,N-dimethylacetamide  
DMAC = Dimethylacetamide  
15 DMAP = 4-(N,N-dimethylamino)pyridine  
DMF = N,N-dimethylformamide  
DSC = disuccinyl carbonate  
EDCI = 1-(3-dimethylaminopropyl)-3-  
ethylcarbodiimide hydrochloride  
20 Et<sub>2</sub>O = diethyl ether  
Et<sub>3</sub>N = triethylamine  
EtOAc = ethyl acetate  
EtOH = ethanol  
FAB MS = fast atom bombardment mass spectroscopy  
25 g = gram(s)  
GIHA HCl = meta-guanidino-hippuric acid  
hydrochloride  
GIHA = meta-guanidino-hippuric acid  
HPLC = high performance liquid chromatography  
30 IBCF = isobutylchloroformate  
K<sub>2</sub>CO<sub>3</sub> = potassium carbonate  
KOH = potassium hydroxide  
LiOH = lithium hydroxide  
MCPBA = m-chloroperoxybenzoic acid or  
35 m-chloroperbenzoic acid  
MeOH = methanol  
MesCl = methanesulfonylchloride  
mg = milligram  
MgSO<sub>4</sub> = magnesium sulfate  
40 ml = milliliter  
mL = milliliter  
MS = mass spectroscopy  
N<sub>2</sub> = nitrogen  
NaCNBH<sub>3</sub> = sodium cyanoborohydride  
45 Na<sub>2</sub>PO<sub>4</sub> = sodium phosphate  
Na<sub>2</sub>SO<sub>4</sub> = sodium sulfate  
NaHCO<sub>3</sub> = sodium bicarbonate  
NaOH = sodium hydroxide  
NH<sub>4</sub>HCO<sub>3</sub> = ammonium bicarbonate  
50 NH<sub>4</sub><sup>+</sup>HCO<sub>2</sub><sup>-</sup> = ammonium formate  
NMM = N-methyl morpholine  
NMR = nuclear magnetic resonance  
RPHPLC = reverse phase high performance liquid  
chromatography  
55 RT = room temperature

KSCN = potassium thiocyanate  
Pd/C = palladium on carbon  
Bn = benzyl  
Et = ethyl  
5 Me = methyl  
Ph = phenyl  
NEt<sub>3</sub> = triethylamine  
t-BOC = tert-butoxycarbonyl  
TFA = trifluoroacetic acid  
10 THF = tetrahydrofuran  
Δ = heating the reaction mixture

As used herein HPLC-Method 1 refers to reverse  
phase C-18 functionalized silica gel column (50 x 300  
15 mm) using a linear gradient of 95% 0.6% TFA/water:5%  
CH<sub>3</sub>CN to 60% 0.6% TFA/water: 40% CH<sub>3</sub>CN with a flow rate  
of 80 ml/minute.

The compounds as shown in Formulas I-III can exist  
in various isomeric forms and all such isomeric forms  
20 are meant to be included. Tautomeric forms are also  
included as well as pharmaceutically acceptable salts  
of such isomers and tautomers.

In the structures and formulas herein, a bond  
drawn across a bond of a ring can be to any available  
25 atom on the ring.

The term "pharmaceutically acceptable salt" refers  
to a salt prepared by contacting a compound of Formula  
I with an acid whose anion is generally considered  
suitable for human consumption. Examples of  
30 pharmacologically acceptable salts include the  
hydrochloride, hydrobromide, hydroiodide, sulfate,  
phosphate, acetate, propionate, lactate, maleate,  
malate, succinate, tartrate salts and the like. All of  
the pharmacologically acceptable salts may be prepared  
35 by conventional means. (See Berge et al., J Pharm.  
Sci., 66(1), 1-19 (1977) for additional examples of  
pharmaceutically acceptable salts.)

For the selective inhibition or antagonism of  $\alpha_v\beta_3$   
integrins, compounds of the present invention may be  
40 administered orally, parenterally, or by inhalation  
spray, or topically in unit dosage formulations

containing conventional pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes, for example, subcutaneous, intravenous, intramuscular, intrasternal, infusion  
5 techniques or intraperitoneally.

The compounds of the present invention are administered by any suitable route in the form of a pharmaceutical composition adapted to such a route, and in a dose effective for the treatment intended.  
10 Therapeutically effective doses of the compounds required to prevent or arrest the progress of or to treat the medical condition are readily ascertained by one of ordinary skill in the art using preclinical and clinical approaches familiar to the medicinal arts.

15 Accordingly, the present invention provides a method of treating conditions mediated by selectively inhibiting or antagonizing the  $\alpha_v\beta_3$  cell surface receptor which method comprises administering a therapeutically effective amount of a compound selected  
20 from the class of compounds depicted in Formulas I-III, wherein one or more compounds of the Formulas I-III is administered in association with one or more non-toxic, pharmaceutically acceptable carriers and/or diluents and/or adjuvants (collectively referred to herein as  
25 "carrier" materials) and if desired other active ingredients. More specifically, the present invention provides a method for inhibition of the  $\alpha_v\beta_3$  cell surface receptor. Most preferably the present invention provides a method for inhibiting bone  
30 resorption, treating osteoporosis, inhibiting humoral hypercalcemia of malignancy, treating Paget's disease, inhibiting tumor metastasis, inhibiting neoplasia (solid tumor growth), inhibiting angiogenesis including tumor angiogenesis, treating diabetic retinopathy,  
35 inhibiting arthritis, psoriasis and periodontal disease, and inhibiting smooth muscle cell migration including restenosis.

Based upon standard laboratory experimental techniques and procedures well known and appreciated by those skilled in the art, as well as comparisons with compounds of known usefulness, the compounds of Formula I can be used in the treatment of patients suffering from the above pathological conditions. One skilled in the art will recognize that selection of the most appropriate compound of the invention is within the ability of one with ordinary skill in the art and will depend on a variety of factors including assessment of results obtained in standard assay and animal models.

Treatment of a patient afflicted with one of the pathological conditions comprises administering to such a patient an amount of compound of the Formula I which is therapeutically effective in controlling the condition or in prolonging the survivability of the patient beyond that expected in the absence of such treatment. As used herein, the term "inhibition" of the condition refers to slowing, interrupting, arresting or stopping the condition and does not necessarily indicate a total elimination of the condition. It is believed that prolonging the survivability of a patient, beyond being a significant advantageous effect in and of itself, also indicates that the condition is beneficially controlled to some extent.

As stated previously, the compounds of the invention can be used in a variety of biological, prophylactic or therapeutic areas. It is contemplated that these compounds are useful in prevention or treatment of any disease state or condition wherein the  $\alpha_v\beta_3$  integrin plays a role.

The dosage regimen for the compounds and/or compositions containing the compounds is based on a variety of factors, including the type, age, weight, sex and medical condition of the patient; the severity of the condition; the route of administration; and the

activity of the particular compound employed. Thus the dosage regimen may vary widely. Dosage levels of the order from about 0.01 mg to about 1000 mg per kilogram of body weight per day are useful in the treatment of  
5 the above-indicated conditions and more preferably of the order from about 0.01 mg to about 100 mg/kg of body weight.

The active ingredient administered by injection is formulated as a composition wherein, for example,  
10 saline, dextrose or water may be used as a suitable carrier. A suitable daily dose would typically be about 0.01 to 100 mg/kg body weight injected per day in multiple doses depending on the factors listed above and more preferably from about 0.01 to about 10 mg/kg  
15 body weight.

For administration to a mammal in need of such treatment, the compounds in a therapeutically effective amount are ordinarily combined with one or more adjuvants appropriate to the indicated route of  
20 administration. The compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanolic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids,  
25 gelatin, acacia, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and tableted or encapsulated for convenient administration. Alternatively, the compounds may be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed  
30 oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

The pharmaceutical compositions useful in the  
35 present invention may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional pharmaceutical adjuvants such

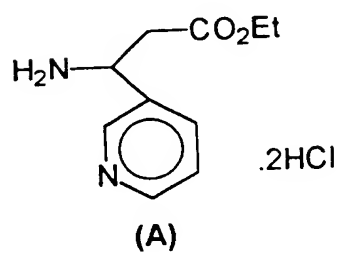
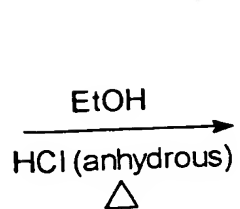
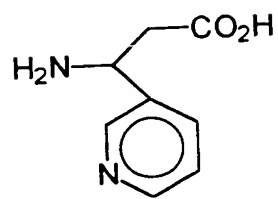
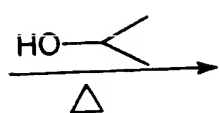
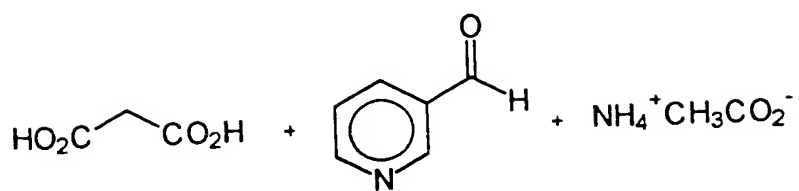


as preservatives, stabilizers, wetting agents, emulsifiers, buffers, etc.

5       The general synthetic sequences for preparing the compounds useful in the present invention are outlined in Schemes I-VI. Both an explanation of, and the actual procedures for, the various aspects of the present invention are described where appropriate. The following Schemes and Examples are intended to be merely illustrative of the present invention, and not  
10   limiting thereof in either scope or spirit. Those of skill in the art will readily understand that known variations of the conditions and processes described in the Schemes and Examples can be used to perform the process of the present invention.

15       Unless otherwise indicated all starting materials and equipment employed were commercially available.

SCHEME I



Schemes I-VI are illustrative of methodology useful for preparing various compounds of the present invention. Such methodology is more specifically defined in the examples which follow. Such methodology  
5 can be modified by one skilled in the art, substituting known reagents and conditions from conventional methodology to produce the desired compounds.

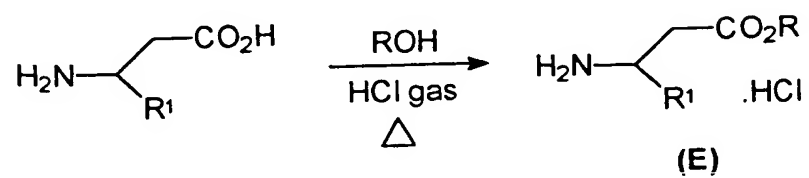
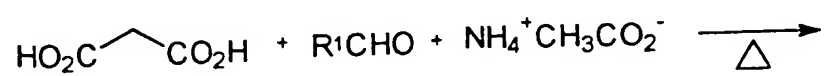
Scheme I describes a synthesis of a pyridyl  $\beta$ -aminoacid which can be used to synthesize compounds of  
10 the present invention wherein  $R^1$  is pyridyl. The reaction can be modified using conventional methodology to prepare other aromatic, alkyl or heterocyclic substituted  $\beta$ -amino acids by substitution of the pyridyl carboxaldehyde with any other appropriate  
15 aldehyde. Briefly, in Scheme I to pyridine-carboxaldehyde in isopropanol is added ammonium acetate followed by malonic acid. The reaction mixture is stirred at reflux, the resulting precipitate filtered and washed with hot isopropanol and dried to yield 3-  
20 amino-3-(3-pyridyl)propionic acid. The ethyl ester is synthesized by heating this acid in excess ethanol in the presence of excess HCl gas.

Additionally,  $\beta$ -amino acids which are useful in the present invention are accessible through modified  
25 Knoevenagel reactions (Secor, H.V.; Edwards, W.B.J. J. Org. Chem. 1979, 44, 3136-40; Bellasoued, M.; Arous-  
Chtar, R.; Gaudemar, M.J.; J. Organometal. Chem. 1982, 231, 185-9), through Reformatski reaction with Schiff bases (Furukawa, M.; Okawara, T.; Noguchi, Y.;  
30 Terawaki, Y. Chem. Pharm. Bull. 1978, 26, 260), Michael addition into an acrylic derivative (Davies, S.G.; Ichihara, O. Tetrahedron:Asymmetry 1991, 2, 183-6; Furukawa, M.; Okawara, TR.; Terawaki, Y. Chem. Pharm. Bull., 1977, 25, 1319-25). More recent methods include  
35 the use of organometallic reagents in Pd or Zn mediated couplings (Konopelski, J.; Chu, K.S.; Negrete, G.R. J. Org. Chem. 1991, 56, 1355; Mokhallalati, M.K.; Wu, M-

J.; Prigden, L.N. Tetrahedron Lett. 1993, 34, 47-50) to complement more traditional reactions such as reductive amination of  $\beta$ -ketoesters.

5 The racemic beta-alkyl beta amino esters can also conveniently be prepared from the corresponding beta lactam by treatment with anhydrous HCl gas in ethanol. The beta lactams were prepared from the corresponding alkene and chlorosulfonyl isocyanate (Szabo, W.A. Aldrichimica Acta, 1977, 23 and references cited  
10 therein). The latter method is useful for the preparation of  $\alpha$  and  $\beta$ -substituted  $\beta$ -aminoacids. (Manhas, M.S.; Wagle, D.R.; Chong, J.; Bose, A.K. Heterocycles, 1988, 27, 1755.) Another route to  $\alpha$ -substituted  $\beta$ -aminoacids is the Raney Nickel reduction  
15 of cyanoacetic esters at temperatures ranging between 20 and 80°C and at 20 to 100 atm pressure (Testa, E.; Fontanella, L.; Fava, F. Farmacol. Ed. Sci., 1958, 13, 152; Testa, E.; Fontanella, L. Annalen 1959, 625, 95). Also, a number of procedures are available for the  
20 preparation of  $\beta$ -aminoacids by reduction of hydrazones of keto-acids (Gottijes, J.; Nomte, W.Th. Rec. Trav. Chem. 1953, 72, 721), oximes (Anziegin, A.; Gulewivich, W. Z. Physiol. Chem., 1926, 158, 32) and nitropropionic acids. Purification of final compounds is usually by  
25 reverse phase high performance liquid chromatography (RP HPLC) [High Performance Liquid Chromatography Protein and Peptide Chemistry, F. Lottspeich, A. Henschler, K.P. Hupa, (eds.) Walter DeGruyter, New York, 1981] or crystallization.

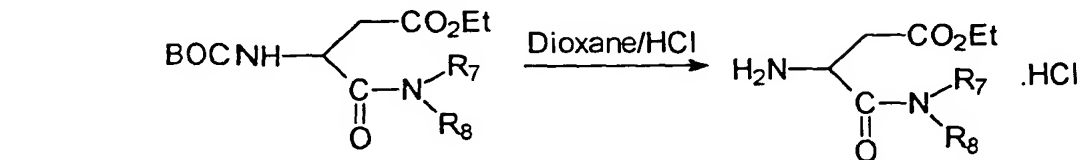
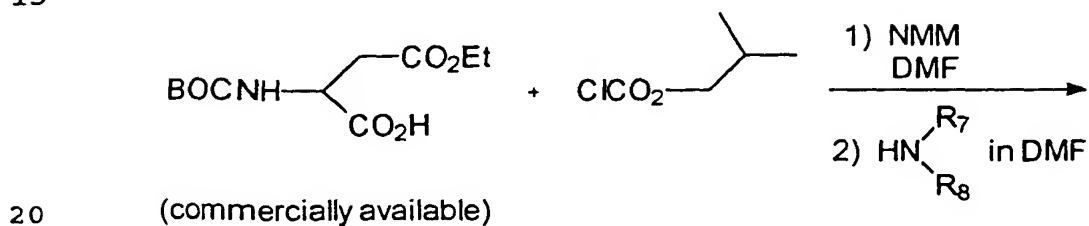
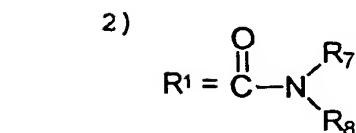
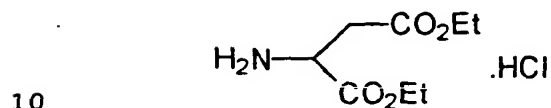
SCHEME II



SCHEME III

For compounds wherein

- 5    1)     $R^1 = CO_2H$   
           (E) is the commercially available



wherein  $\text{HN} \begin{array}{l} \nearrow \text{R}_7 \\ \searrow \text{R}_8 \end{array}$  denotes an amino acid, the amino acid

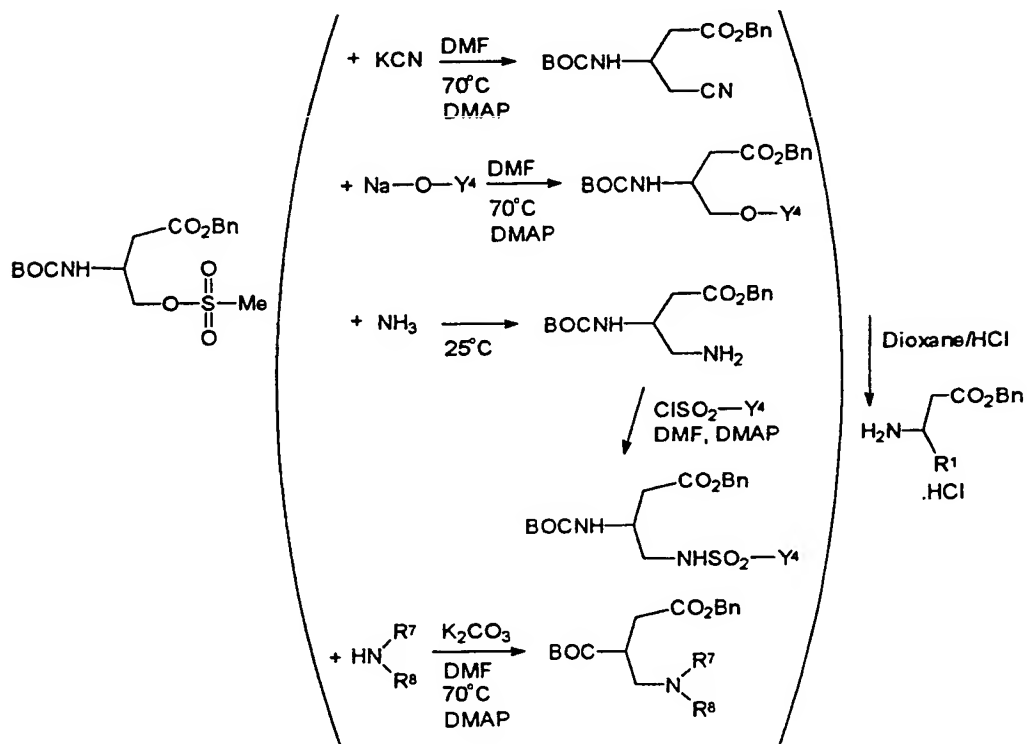
being protected with the appropriate protecting groups.

Additional methodologies for further  $R^1$  groups are as follows:


$$\begin{array}{c} \text{---CH}_2\text{S---Y}^4 \\ \quad \parallel \\ \quad \text{O} \\ \text{---CH}_2\text{SO}_2\text{---} \end{array}$$

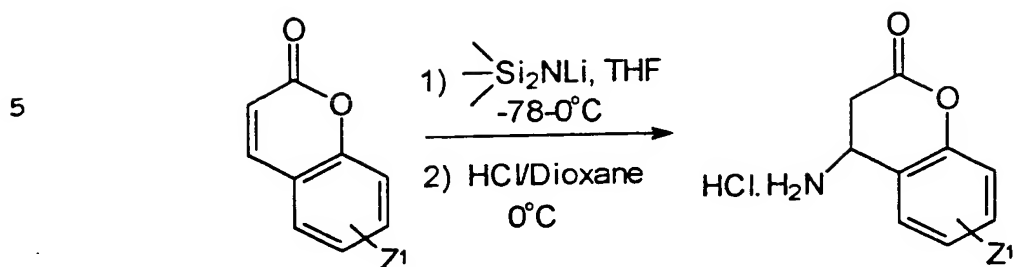
SCHEME III (Cont'd)

In a similar manner, compounds of the present invention wherein R<sup>1</sup> is substituted alkyl can be synthesized in the following manner:



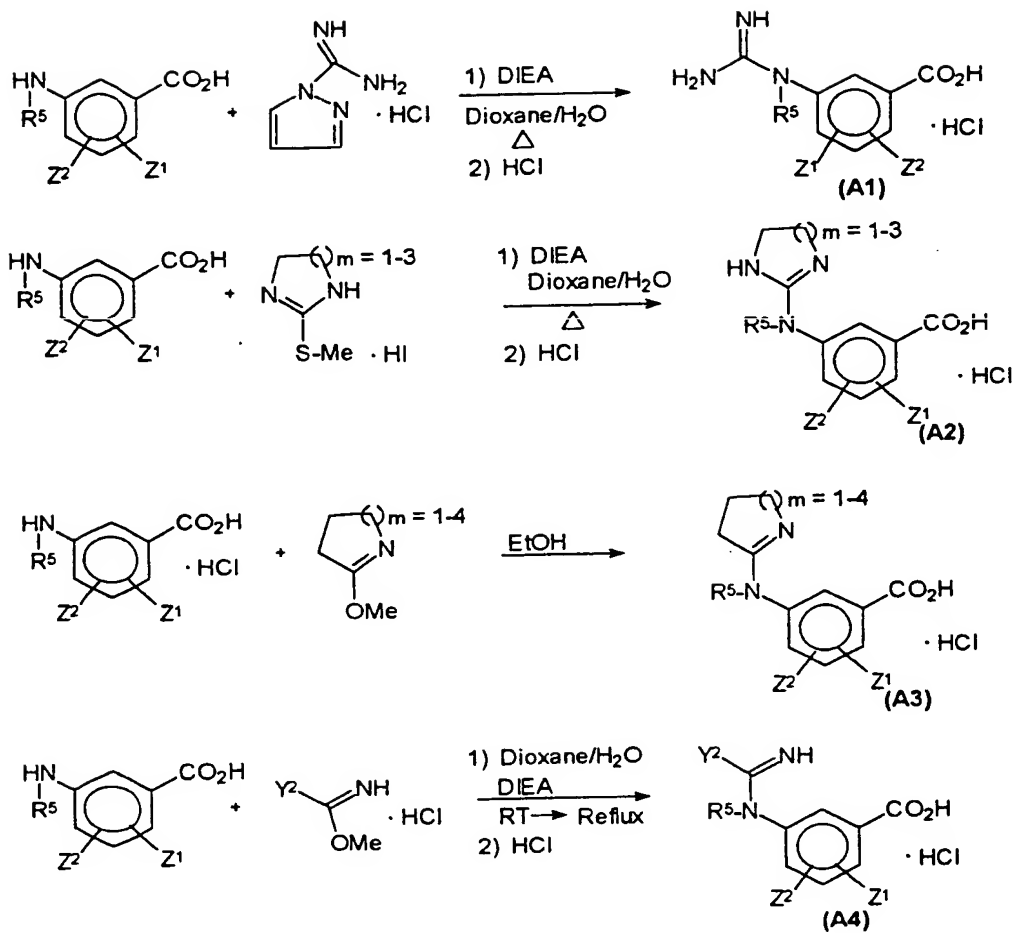


SCHEME IV

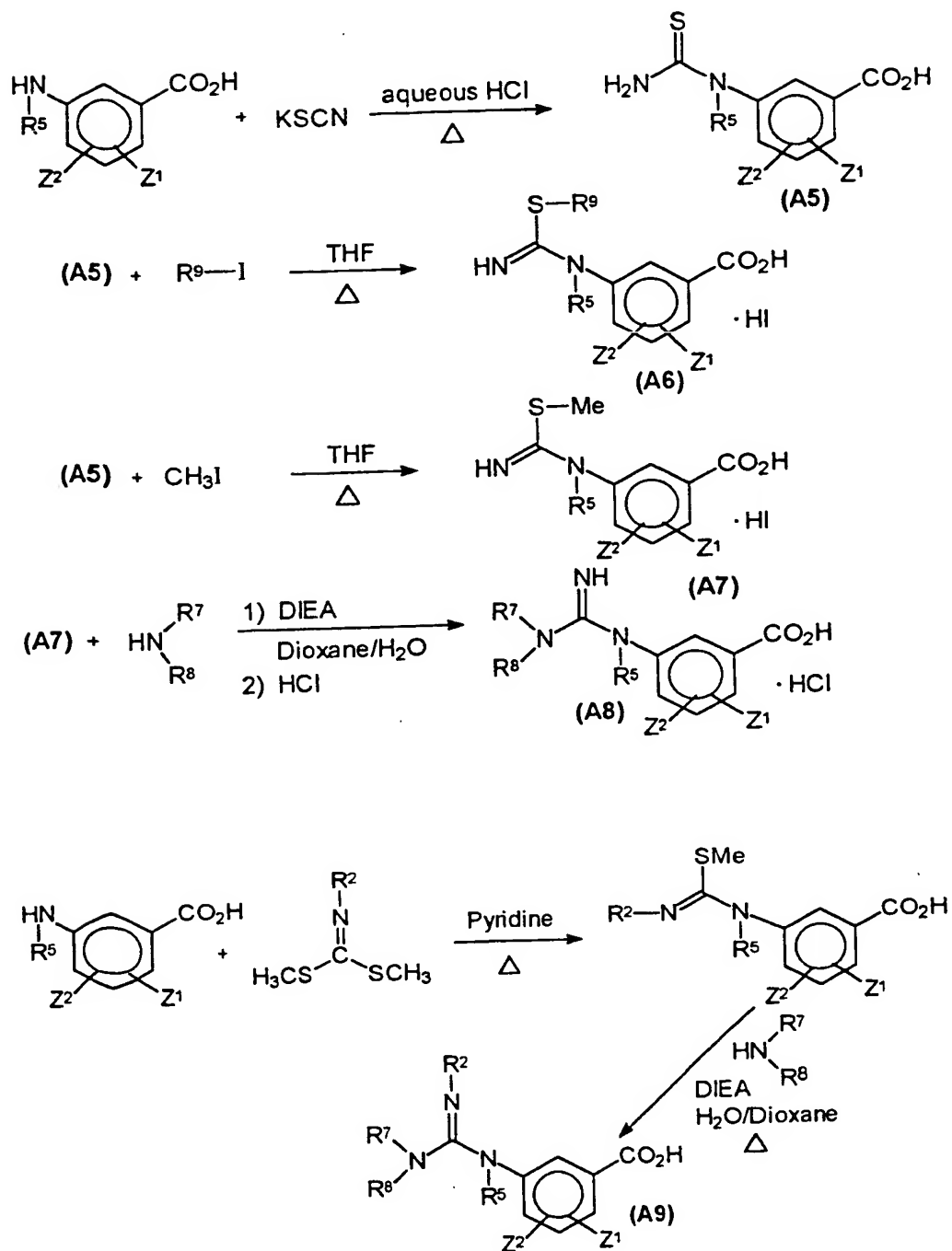


Scheme IV represents the synthesis of aminohydrocoumarins (see J. Rico, Tett. Let., 1994, 35, 6599-6602) which are readily opened to form R<sup>1</sup> being an orthohydroxyphenyl moiety, further substituted by Z<sup>1</sup>.

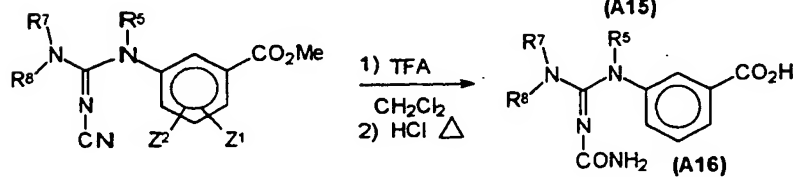
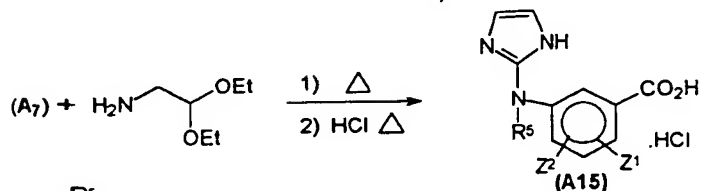
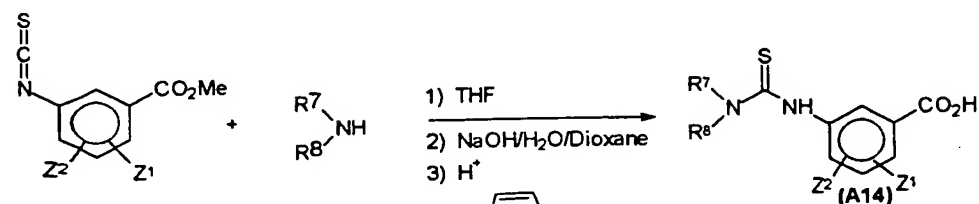
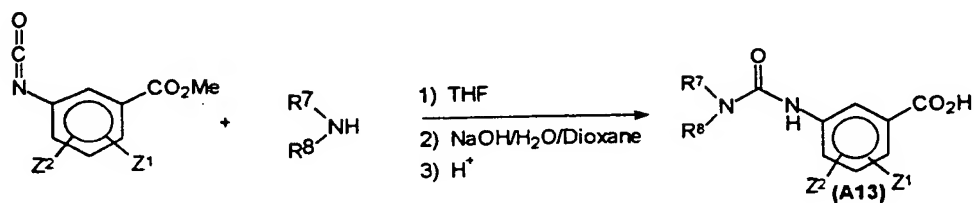
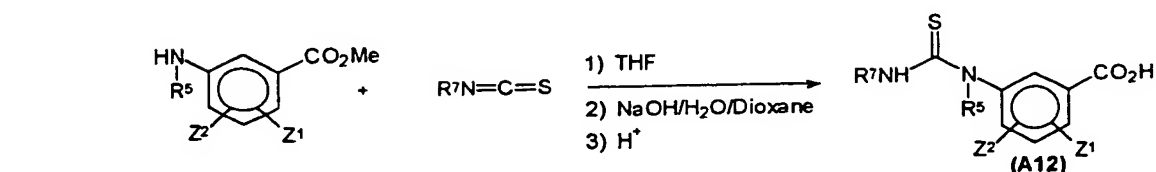
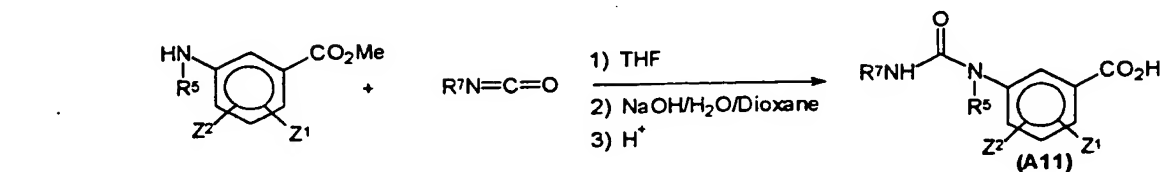
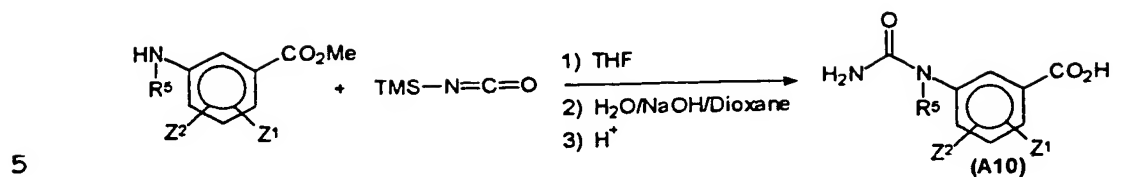
SCHEME V



SCHEME V (Cont'd)



SCHEME V (Cont'd)



Specifically, in Scheme V:

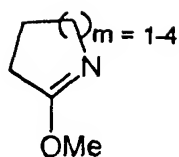
In the synthesis of intermediate benzoic acids  
(A1) through (A16), the starting amino benzoic acids



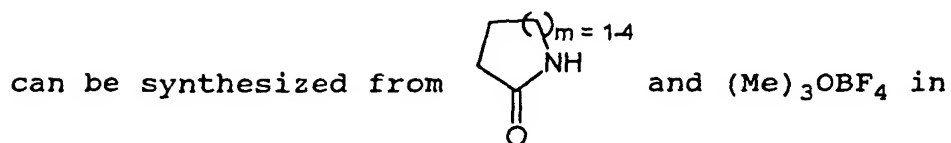
can be converted to such amino benzoic acids via  
reduction of the corresponding nitro benzoic acid,  
which can be obtained commercially or synthesized by  
nitration of the appropriate benzoic acid, followed by  
10 reduction to the desired amino benzoic acid. These are  
all when R<sup>5</sup> is H. If R<sup>5</sup> is other than H, alkylation of  
the amino functionality can be achieved by conventional  
methodology.

Furthermore, synthesis of intermediate (A2) can  
15 also be accomplished as disclosed generally in US  
3,202,660, starting with the appropriate amino benzoic  
acid. Furthermore, intermediate (A2) and (A15) as well  
as further analogues of (A2) and (A15) such as  
substitutions on the heterocyclic ring, oxazolidines,  
20 thiazolidines, benzimidazoles and the like can also be  
accomplished as disclosed in

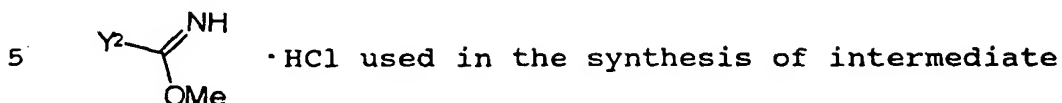
- 1) Chem. Pharm. Bull. 41(1) 117-125 (1993)
- 2) Chem. Pharm. Bull. 33(10) 4409-4421 (1985)
- 3) J. Med. Chem. 18 (1), 90-99 (1975).



used in the synthesis of intermediates (A3),



dichloromethane.



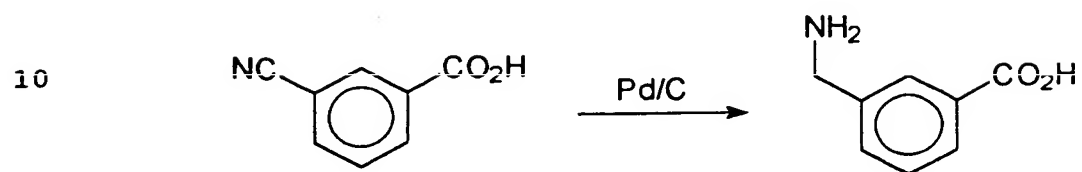
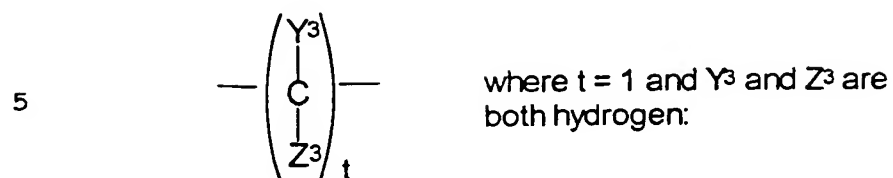
(A4), can be synthesized from Y<sup>2</sup>-CN and MeOH (1 equivalent) and HCl gas (1 equivalent) in heptane.

All other reagents in Scheme I are either commercially available or readily synthesized by methodologies known by those skilled in the art.

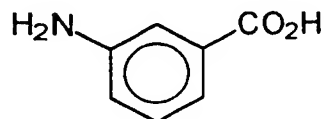
10 When R<sup>50</sup> is not H, the appropriate nitrogen can be alkylated in an appropriate step by methodology known to those skilled in the art. Alternate acid derivatives R are synthesized by methodologies known to those skilled in the art.

15

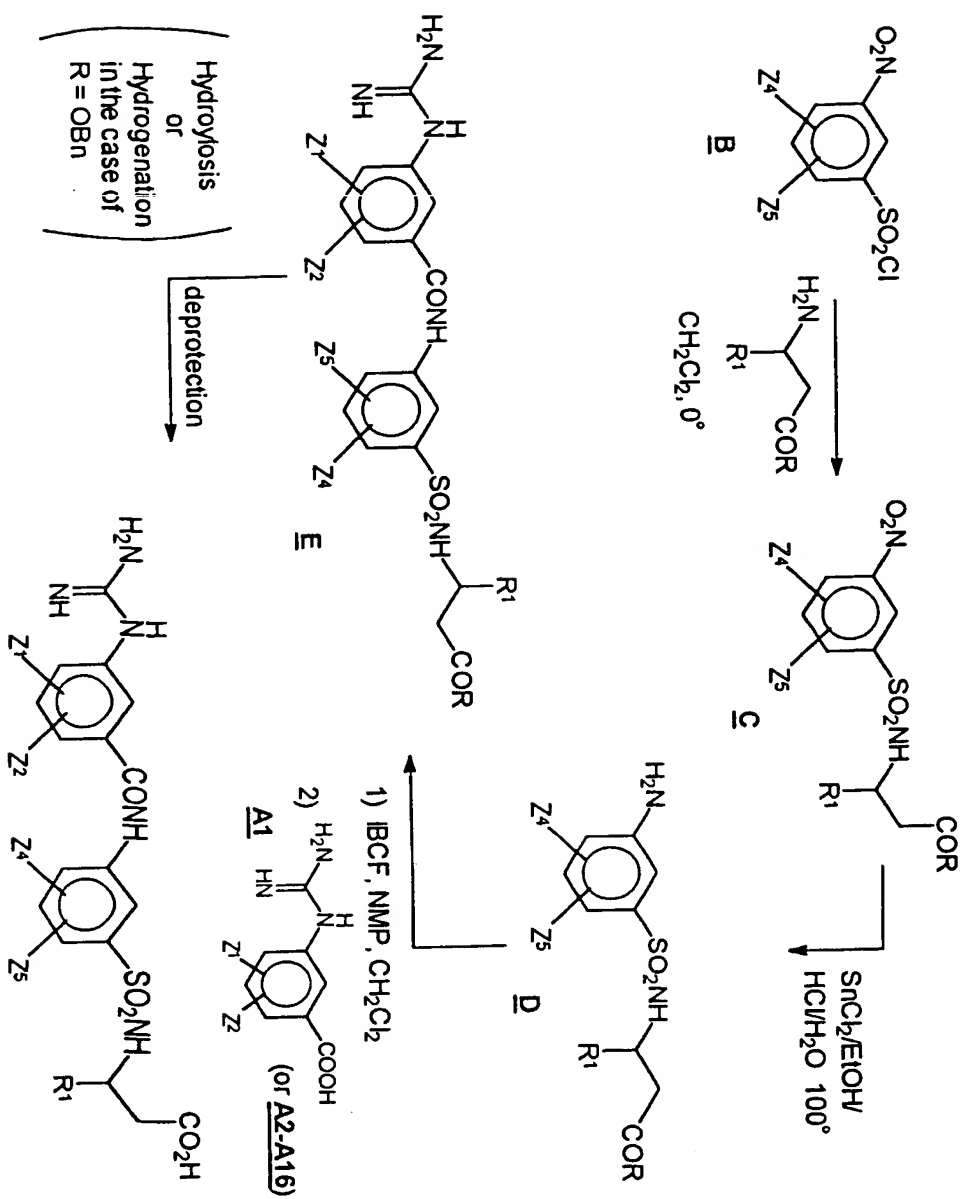
To synthesize compounds wherein



15 which is then treated in the same manner of further  
derivatization as exemplified in the previous schemes  
for:



SCHEME VI



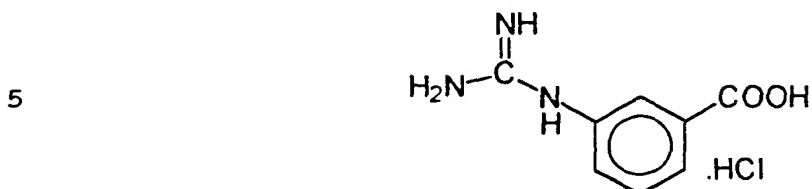


Compounds of the present invention may be prepared as follows:

3-Nitrophenylsulphonylchloride B can be coupled to  $\beta$ -amino acids (as prepared in Schemes I-IV) to afford adduct C. Reduction of C ( $\text{SnCl}_2$ , EtOH, HCl,  $\text{H}_2\text{O}$ ,  $100^\circ$ ) affords aniline D. Aniline D can be coupled to intermediates (A1-16) as prepared in Scheme V using well known and standard coupling procedures, followed by hydrolysis (or deprotection) of the resulting ester to afford compounds of the present invention.

Example A

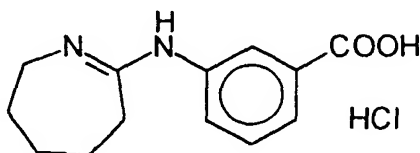
(3-Guanidinobenzoic acid hydrochloride)



To 3,5-dimethylpyrazole-1-carboxamidine nitrate  
(6 g, 0.03 mole) (Aldrich) and diisopropylamine (3.8 g,  
10 0.03 mole) in dioxane (20 ml) and H<sub>2</sub>O (10 ml) was added  
3-aminobenzoic acid (2.7 g, 0.02 mole). The reaction  
was stirred at reflux for 2.5 hours then overnight at  
room temperature. The resulting precipitate was  
filtered, washed with dioxane/H<sub>2</sub>O and dried. The  
15 precipitate was then slurried in H<sub>2</sub>O and acidified with  
concentrated HCl until a solution formed. The solvent  
was removed under vacuum and the residue was slurried  
twice in ether (ether decanted off). The product was  
dried under vacuum to yield 3-guanidinobenzoic acid  
20 hydrochloride (1.77 g) as a white solid. MS and NMR  
were consistent with the desired structure.

Example B

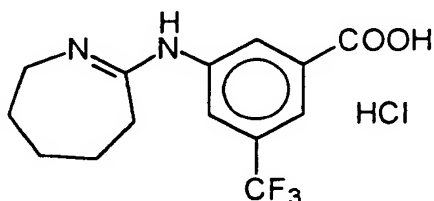
3-(1-Aza-2-amino-1-cycloheptenyl)benzoic acid  
25 hydrochloride



30 To 1-aza-2-methoxy-1-cycloheptene (3.67 g, 0.0288  
mole) (Aldrich) in absolute ethanol (20 ml) was added  
3-aminobenzoic acid hydrochloride (5 g, 0.0288 mole).  
A solution quickly formed. The reaction mixture was  
stirred overnight at room temperature. The resulting  
35 precipitate was filtered, washed with ether and dried  
under vacuum to yield 3-(1-aza-2-amino-1-cycloheptene)-  
benzoic acid (4.9 g).

Example C

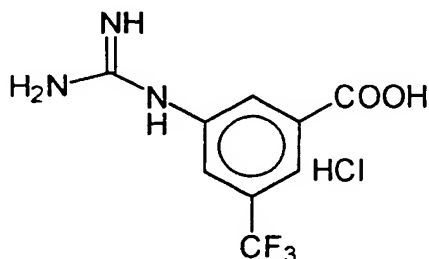
3-(1-aza-2-amino-1-cycloheptenyl)-5-trifluoromethylbenzoic acid hydrochloride



The title compound was synthesized according to the methodology of Example B, substituting an equivalent amount of 3-amino-5-trifluoromethyl benzoic acid [which was synthesized by reduction of 3-nitro-5-trifluoromethyl benzoic acid (Lancaster) in ethanol with 10% Pd/C under 50 psi H<sub>2</sub> for 4 hours] for 3-aminobenzoic acid.

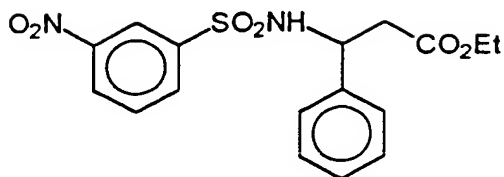
Example D

3-guanidino-5-trifluoromethylbenzoic acid, hydrochloride



The title compound was synthesized according to the methodology of Example A, substituting an equivalent amount of 3-amino-5-trifluoromethylbenzoic acid (see Example C) for 3-aminobenzoic acid.

Example E

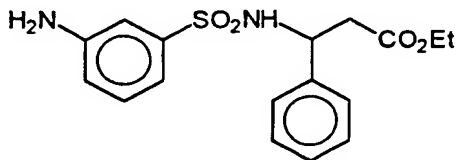


5

In a dried flask under nitrogen at 0° was dissolved 3-nitrobenzene sulfonyl chloride (2.2 g) (Aldrich) in methylene chloride (25 ml). A solution of  
10  $\beta$ -phenyl alanine ethyl ester hydrochloride (2.3 g), triethylamine (2.3 g) and methylene chloride (25 ml) was added at a rate so as not to allow the temperature to rise above 20°. The reaction mixture was stirred at room temperature for 1 hour and then partitioned  
15 between methylene chloride and water. The aqueous portion was extracted several times with additional methylene chloride and the combined organic extracts were washed with saturated sodium chloride solution, dried (Na<sub>2</sub>SO<sub>4</sub>), concentrated and purified on a silica  
20 gel column eluting with 40% ethyl acetate -60% hexane to afford 3.3 g of white solid.

NMR was consistent with the proposed structure.

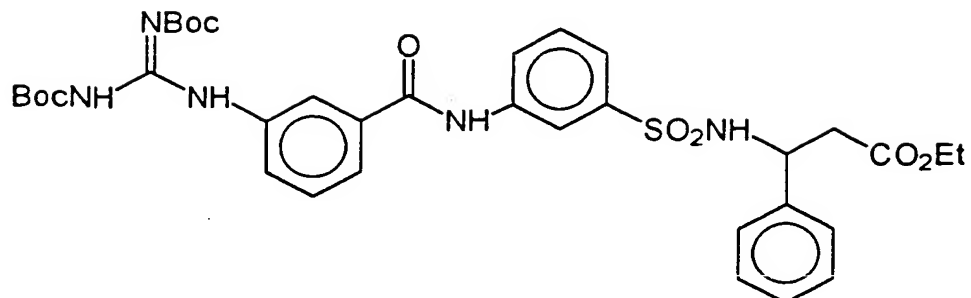
Example F



25

A solution of the product from Example E (3.2 g) in dimethyl formamide (30 ml) was hydrogenated under a  
30 hydrogen atmosphere at room temperature for 16 hours using 4% palladium on carbon (300 mg). The reaction mixture was concentrated and purified on a silica gel column using 1:1 ethyl acetate:hexane as eluant to afford 1.6 g of a viscous golden oil. NMR was  
35 consistent with the proposed structure.

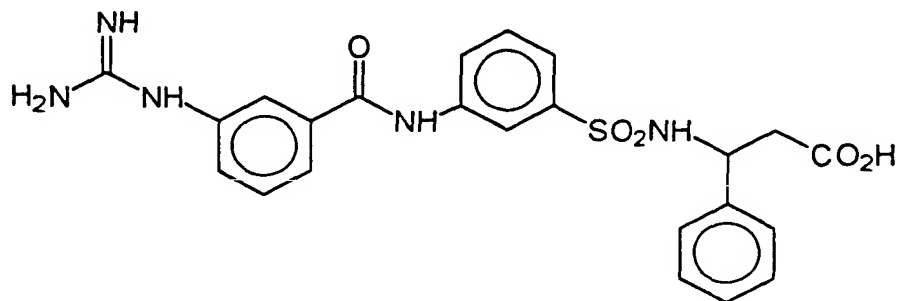
Example G



- 5            To a solution of 3-bis-boc-guanidine benzoic acid (266 mg) and N-methylmorpholine (76 mg) (Fluka) in DMF (3 ml) at 0° under nitrogen was added a solution of isobutylchloroformate (96 mg) (Aldrich) in DMF (2 ml) in one portion.
- 10           The reaction mixture was stirred for 30 minutes and then a solution of the product from Example F (250 mg) and DMF (2 ml) was added in one portion. The reaction mixture was stirred at room temperature for 2 days and then the solvent was removed *in vacuo*. The
- 15           residue was purified on a silica gel column using 30% ethyl acetate -70% hexane as eluant to afford 95 mg of white solid. NMR was consistent with the proposed structure.

Example 1

Synthesis of  $\beta$ -[[[3-[[[3-[(aminoiminomethyl)amino]  
phenyl]carbonyl]amino]phenyl]sulfonyl]amino]-  
5 benzenepropanoic acid, trifluoroacetate salt



A solution of the product from Example G (90 mg),  
15 1,4 dioxane (2.5 ml) and 6N hydrochloric acid (2.5 ml)  
was stirred at room temperature for 17 hours. The  
solvent was removed in vacuo and the residue was  
purified via reverse phase HPLC using a water (0.5%  
TFA) and acetonitrile gradient as eluant to afford 64  
20 mg of white solid. NMR was consistent with the  
proposed structure.

Analysis Calculated for  $C_{23}H_{23}N_5O_5S \cdot 2.5 CF_3CO_2H$

C, 43.87; H, 3.35; N, 9.14; S, 4.18.

25 Found: C, 43.45; H, 3.30; N, 9.16; S, 4.47.

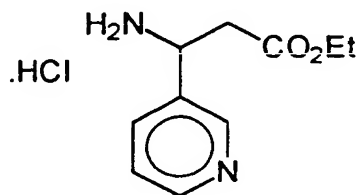
The compounds of this invention and the following Examples 2-9 were prepared according to the methodology that follows:

5

Example H

General procedure for the following amino esters:

10



15

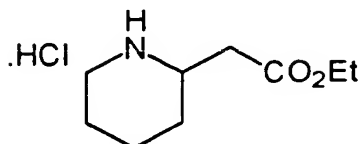
A slurry of 3-pyridinecarboxaldehyde (5.0 gm, 46.7 mmol), malonic acid (5.83 gm, 56 mmol), and ammonium acetate (4.32 gm, 56 mmol) in isopropanol (50 mL) was heated to reflux under a nitrogen atmosphere for 2-3 hours. The reaction mixture was cooled to room temperature and the solids collected by vacuum filtration. The solids were washed on the filter with hot isopropanol (50 mL) and diethyl ether (50 mL) and then dried overnight under vacuum. The crude acid was dissolved in ethanol (50 mL) and anhydrous hydrogen chloride gas was passed through the ethanol solution for 30 minutes. The reaction mixture was then concentrated in vacuo and the remaining white solids were triturated with diethyl ether (50 mL). The white solids were collected and dried under vacuum to afford 5.85 gm (70%) of the amino ester. <sup>1</sup>H NMR was consistent with the expected product.

30

Example I

Procedure for the following amino ester:

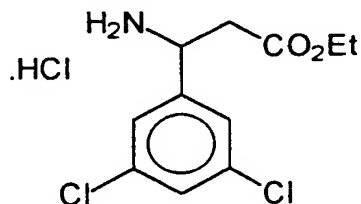
5



10 2-Pyridylacetic acid hydrochloride (10 gm, 57.6 mmol) was subjected to hydrogenation conditions (PtO<sub>2</sub> in AcOH solvent, 60 psi, 40°C) to afford the piperidyl product 8.0 gm (80%). The resulting amino acid was subjected to the above esterification conditions (Example H) to afford 8.32 gm (90%) of product. <sup>1</sup>H NMR  
15 was consistent with the expected product.

Example J

20

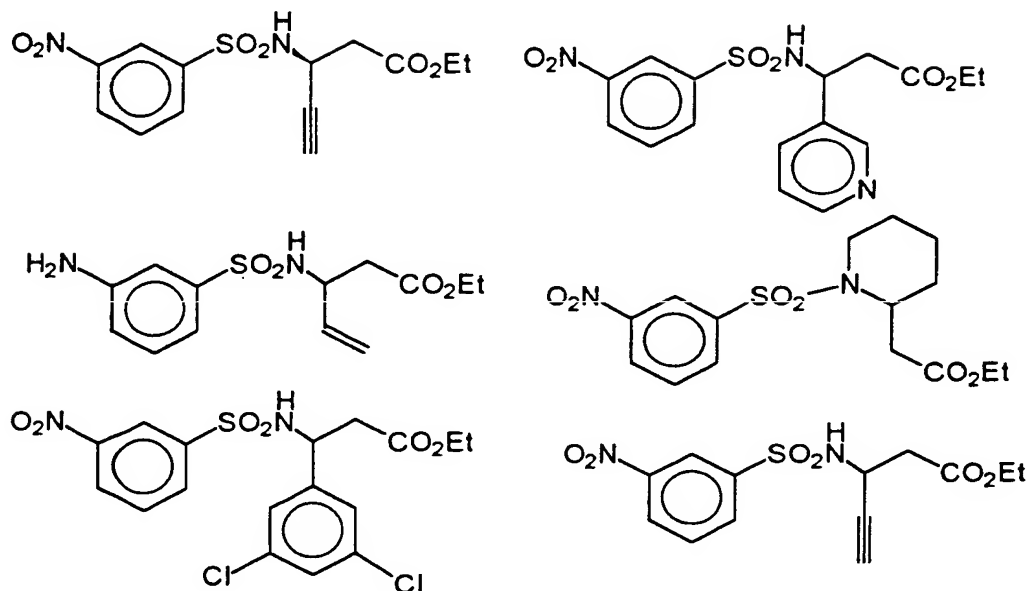


25 The above compound was prepared using the methodology described in Example H.



Example L

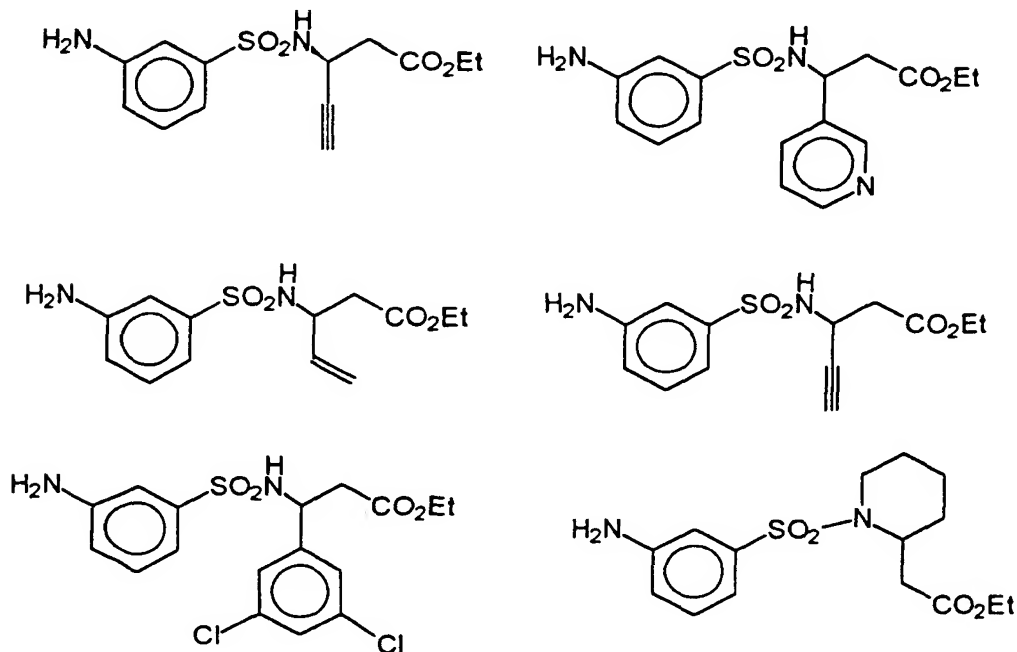
General procedure for the following aryl nitro compounds:



A solution of 3-nitrobenzenesulfonyl chloride  
5 (0.65 gm, 2.93 mmol) and the corresponding amino ester  
prepared via methodology described in Examples H-J  
[prepared as described in J. Med. Chem., 1995, 38, 2378  
or commercially available] in methylene chloride (10  
mL) was cooled to 0°C under a nitrogen atmosphere. To  
10 the cooled suspension was then added triethylamine  
(0.82 mL, 5.86 mmol) and the reaction mixture was  
allowed to stir at 0°C for 1 hour, then warmed to room  
temperature for 2 hours. The reaction mixture was then  
transferred to a separatory funnel and diluted with 20  
15 mL water. After extraction, the isolated aqueous layer  
was reextracted with methylene chloride (20 mL). The  
combined organic extracts were washed with brine (25  
mL), dried over MgSO<sub>4</sub>, vacuum filtered, and  
concentrated in vacuo to afford a crude white solid.  
20 The solids were triturated with 25% ethyl acetate in  
hexanes (50 mL) and the resulting white crystals were  
collected and dried overnight under vacuum. Final  
yield of product was 0.76 gm (79%). <sup>1</sup>H NMR was  
consistent with the expected product.

Example M

General procedure for the following sulfonamides:



5

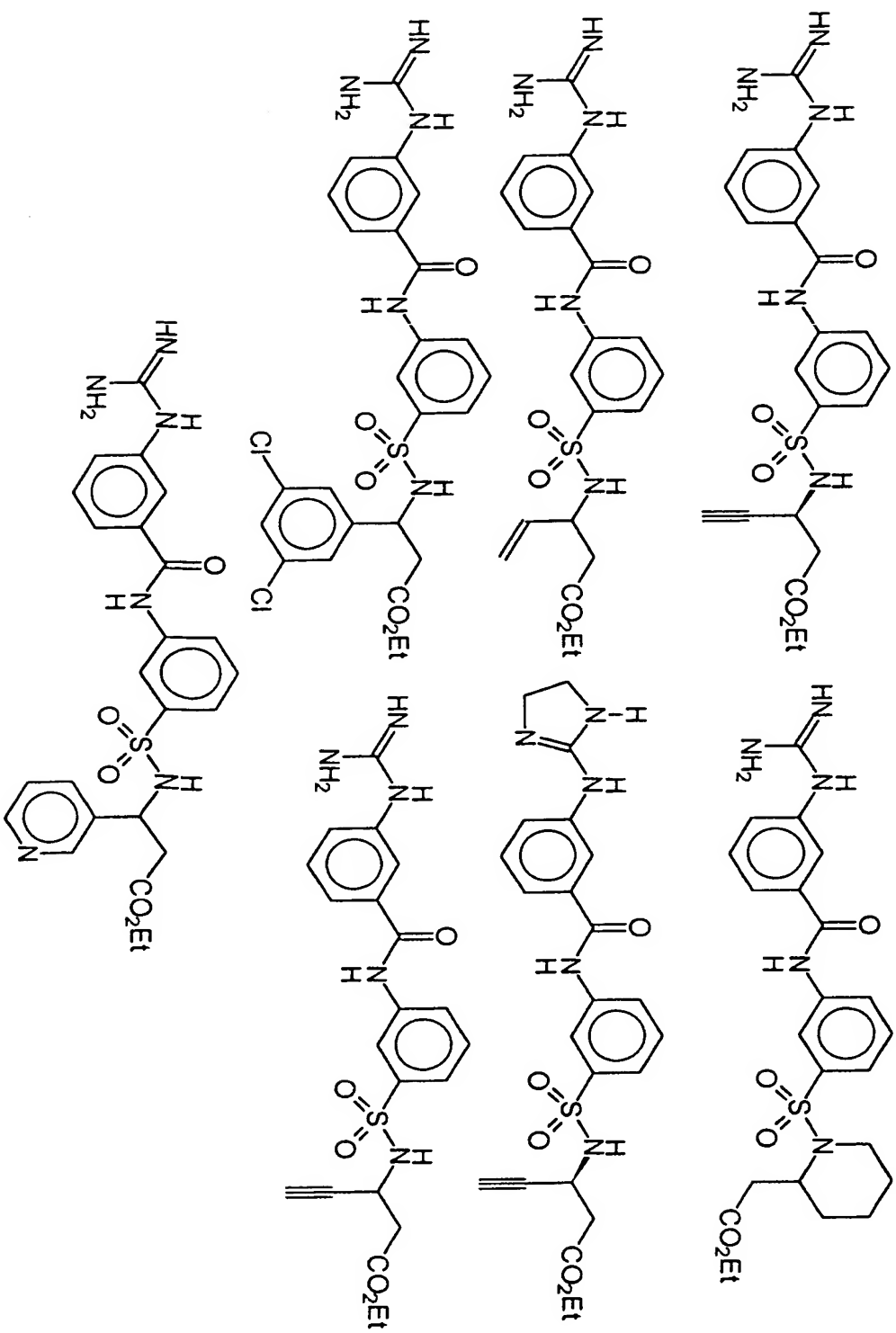
A solution of the nitro sulfonamide from Example L (0.40 gm, 1.23 mmol) and tin (II) chloride·2 H<sub>2</sub>O in ethanol (25 mL) was heated to 80°C under nitrogen for 2 hours. After cooling to room temperature the reaction mixture was poured into ice water (40 mL) and brought to a basic pH by the slow addition of saturated sodium bicarbonate solution (40 mL). The resulting mixture was then extracted twice with ethyl acetate (2 x 30 mL). The combined organic extracts were then dried over MgSO<sub>4</sub>, vacuum filtered, and concentrated *in vacuo* to afford the amino sulfonamide as an oily product (0.36 gm, 95%). No further purification was necessary. <sup>1</sup>H NMR was consistent with the expected product.

10

15

Example N

General procedure for the following esters:



A solution of the compound from Example A (240 mg, 1.11 mmol) in DMAC (5 mL) was cooled to -10°C under a nitrogen atmosphere. To this solution was then added in sequence isobutyl chloroformate (0.15 mL, 1.17 mmol) followed by N-methyl morpholine (0.13 mL, 1.17 mmol). The resulting mixture was allowed to stir for 30 minutes at -10°C. In a separate flask the sulfonamide was dissolved in DMAC (5 mL) and then transferred to the reaction mixture via syringe. The resulting solution was allowed to warm to room temperature while stirring for 18 hours. The reaction mixture was concentrated *in vacuo* and the crude product was purified by HPLC to afford the above ester (45%, 284 mg). <sup>1</sup>H NMR was consistent with the expected product.

The compounds of Examples 2-8 were prepared in the following manner.

A solution of the ethyl ester from Example N (230 mg, 0.40 mmol) in methanol (2 mL), THF (2 mL), and 1 N NaOH (5 mL) was stirred for 2 hours at 20°C. The reaction mixture was then concentrated *in vacuo* to afford a white residue. Purification of the crude product by HPLC (Method 1) afforded the acid as a white crystalline solid.

10 Yields:

Example 2 55%

Example 3 57%

Example 4 73%

Example 5 45%

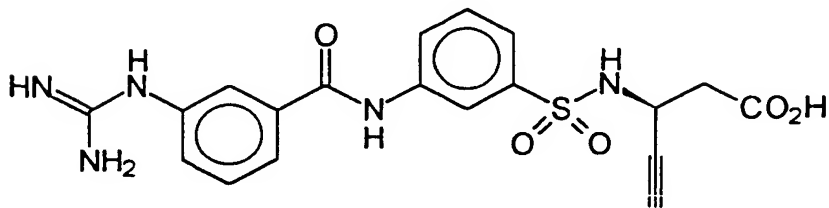
15 Example 6 52%

Example 7 58%

Example 8 60%

Example 2

(±) 3-[[[3-[[[3-[(aminoiminomethyl)amino]phenyl]-  
carbonyl]amino]phenyl]sulfonyl]amino]-4-  
pentynoic acid



<sup>1</sup>H NMR (DMSO) δ 12.25 (s, 1H), 10.28 (s, 1H), 10.0 (s, 1H), 8.18 (d, 1H), 8.16 (s, 1H), 8.03 (d, 1H), 7.9 (d, 1H), 7.8 (s, 1H), 7.6 (m, 5H), 7.45 (d, 1H), 4.25 (m, 1H), 3.45 (m, 1H), 3.05 (s, 1H), 2.58 (d, 2H).  
<sup>13</sup>C NMR (DMSO) 164.8, 155.8, 144.4, 139.4, 135.7, 129.9, 129.5, 127.8, 125.6, 123.7, 121.9, 118.2, 74.7, 41.8, 41.4, 40.0 Hz.

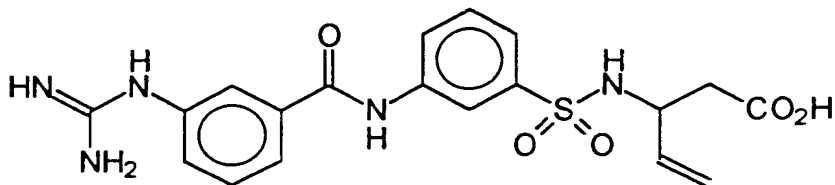
Analysis Calc'd for C<sub>19</sub>H<sub>19</sub>N<sub>5</sub>O<sub>5</sub>S·1.15 TFA

C, 46.41; H, 3.71; N, 12.89

Found: C, 45.67; H, 3.59; N, 12.40

Example 3

(±) 3-[[[3-[[[3-[(aminoiminomethyl)amino]phenyl]-  
carbonyl]amino]phenyl]sulfonyl]amino]-4-  
pentenoic acid, trifluoroacetate salt



10

<sup>1</sup>H NMR (DMSO) δ 12.25 (s, 1H), 10.57 (s, 1H), 10.02 (s, 1H), 8.33 (s, 1H), 8.0 (d, 1H), 7.97 (d, 1H), 7.86 (s, 1H), 7.60 (m, 5H), 7.58 (t, 1H), 7.55 (t, 1H), 7.46 (d, 1H), 5.55 (m, 1H), 4.95 (d, 1H), 4.89 (d, 1H), 4.05 (m, 1H), 2.38 (m, 2H).

15

<sup>13</sup>C NMR (DMSO) 171.2, 164.8, 155.8, 142.1, 139.4, 137.0, 135.7, 129.9, 129.5, 127.8, 125.6, 123.7, 123.5, 121.6, 118.1, 115.7 Hz.

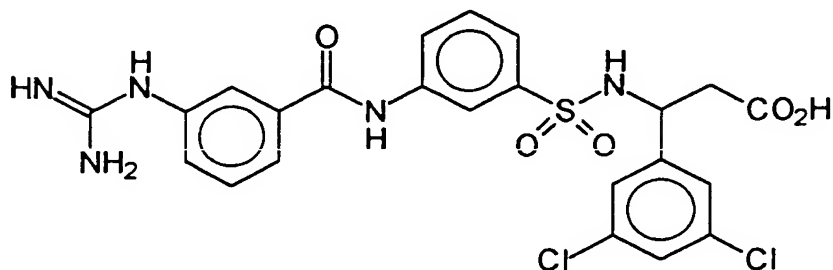
20 Analysis Calc'd for C<sub>19</sub>H<sub>21</sub>N<sub>5</sub>O<sub>5</sub>S·1.3 TFA

C, 44.75; H, 3.88; N, 12.08

Found: C, 44.94; H, 3.65; N, 12.07

Example 4

(±) β-[[[3-[[[3-[(aminoiminomethyl)amino]phenyl]-  
carbonyl]amino]phenyl]sulfonyl]amino]-3,5-  
dichlorobenzene propanoic acid,  
trifluoroacetate salt



<sup>1</sup>H NMR (DMSO) δ 10.40 (s, 1H), 9.80 (s, 1H), 8.43 (d, 1H), 8.10 (s, 1H), 7.90 (d, 1H), 7.85 (d, 1H), 7.60 (t, 1H), 7.50 (m, 5H), 7.39 (t, 1H), 7.30 (d, 1H), 7.22 (t, 1H), 7.14 (d, 2H), 4.62 (m, 1H), 2.60 (m, 2H).

<sup>13</sup>C NMR (DMSO) 170.63, 164.65, 155.8, 144.0, 141.0, 139.3, 135.8, 135.6, 133.6, 129.8, 129.0, 127.8, 126.7, 125.7, 123.8, 123.1, 117.8, 53.9, 41.8 Hz.

Analysis Calc'd for C<sub>23</sub>H<sub>21</sub>Cl<sub>2</sub>N<sub>5</sub>O<sub>5</sub>S·1.2 TFA

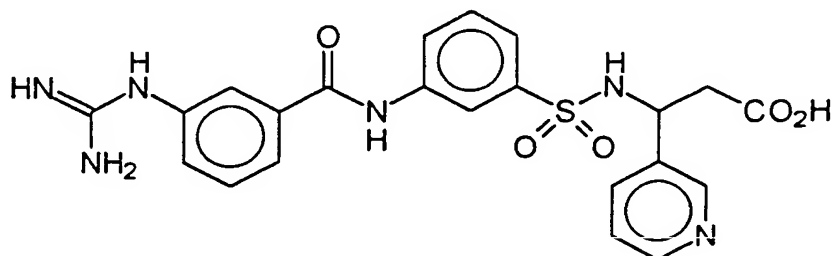
C, 44.39; H, 3.26; N, 10.19

Found: C, 44.39; H, 2.92; N, 10.19



Example 5

$\beta$ -[[[3-[[[3-[(aminoiminomethyl)amino]phenyl]-  
carbonyl]amino]phenyl]sulfonyl]amino]pyridine-3-  
propanoic acid, tris(trifluoroacetate) salt



$^1\text{H}$  NMR (DMSO)  $\delta$  10.22 (s, 1H), 10.0 (s, 1H), 8.58 (d, 1H), 8.45 (s, 1H), 8.42 (d, 1H), 8.15 (s, 1H), 7.90 (d, 1H), 7.85 (m, 2H), 7.60 (m, 4H), 7.47 (d, 1H), 7.37 (m, 3H), 4.75 (m, 1H), 2.77 (m, 2H).  
 $^{13}\text{C}$  NMR (DMSO) 170.6, 164.7, 155.8, 141.4, 139.3, 135.7, 129.9, 129.4, 127.8, 125.6, 124.3, 123.4, 121.4, 118.0, 52.1, 41.4 Hz.

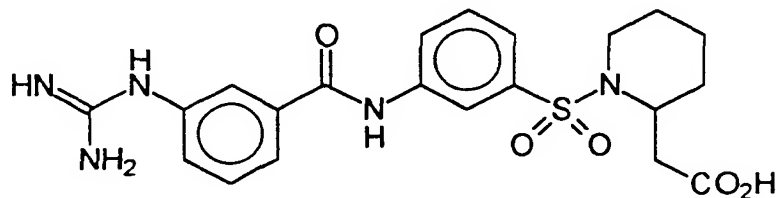
Analysis Calc'd for  $\text{C}_{22}\text{H}_{23}\text{N}_6\text{O}_5\text{S} \cdot 3.0 \text{ TFA} \cdot 1.0 \text{ H}_2\text{O}$

C, 39.87; H, 3.35; N, 9.96

Found: C, 39.84; H, 3.02; N, 10.24

Example 6

1-[[3-[[[3-[(aminoiminomethyl)amino]phenyl]carbonyl]-  
amino]phenyl]sulfonyl]piperidine-2-acetic acid,  
trifluoroacetate salt



10

$^1\text{H}$  NMR (DMSO)  $\delta$  10.58 (s, 1H), 10.03 (s, 1H), 8.30 (s, 1H), 8.06 (d, 1H), 7.90 (d, 1H), 7.85 (s, 1H), 7.60 (m, 5H), 7.56 (d, 1H), 7.52 (d, 1H), 7.45 (d, 1H), 4.35 (m, 1H), 3.0 (t, 1H), 2.7 (dd, 1H), 2.28 (dd, 1H), 1.45 (m, 6H), 1.15 (2H).

15

Analysis Calc'd for  $\text{C}_{21}\text{H}_{25}\text{N}_5\text{O}_5\text{S} \cdot 1.4 \text{ TFA}$

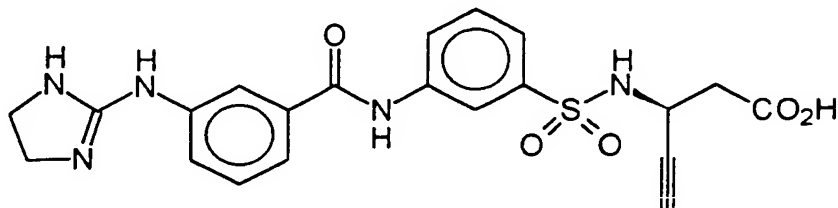
C, 46.17; H, 4.30; N, 11.31

Found: C, 46.02; H, 4.30; N, 11.32

Example 7

3S-[[[3-[[[3-[(4,5-dihydro-1H-imidazol-2-yl)amino]-  
phenyl]carbonyl]amino]phenyl]sulfonyl]amino]-4-  
pentynoic acid, trifluoroacetate salt

5



10

<sup>1</sup>H NMR

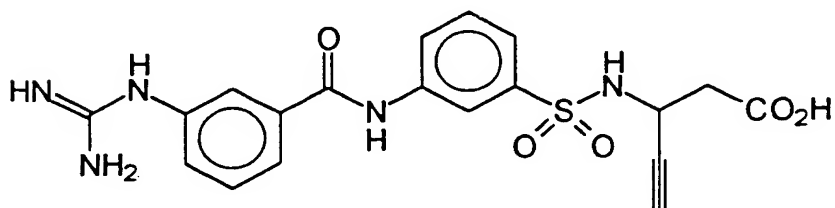
(DMSO) δ 12.53 (s, 1H), 10.7 (s, 1H), 10.6 (s, 1H),  
8.49 (s, 2H), 8.38 (d, 1H), 8.34 (s, 1H), 8.06 (d, 1H),  
7.91 (d, 1H), 7.86 (s, 1H), 7.63 (d, 1H), 7.59 (d, 1H),  
15 7.57 (s, 4H), 7.47 (d, 1H), 4.28 (m, 1H), 3.02 (s, 1H),  
2.58 (d, 2H).

Analysis Calc'd for C<sub>21</sub>H<sub>21</sub>N<sub>5</sub>O<sub>5</sub>S·1.3 TFA

20 C, 46.95; H, 3.72; N, 11.60  
Found: C, 46.87; H, 3.61; N, 11.83

Example 8

3-[[[3-[[[3-[(aminoiminomethyl)amino]phenyl]-  
carbonyl]amino]phenyl]sulfonyl]amino]-4-  
pentynoic acid, trifluoroacetate salt

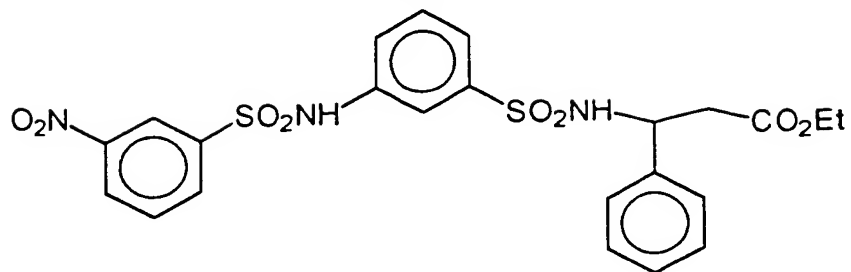


Analysis Calc'd for  $C_{19}H_{19}N_5O_5S \cdot 1.3$  TFA

C, 44.91; H, 3.54; N, 12.12

Found: C, 44.90; H, 3.40; N, 12.34

Example AA

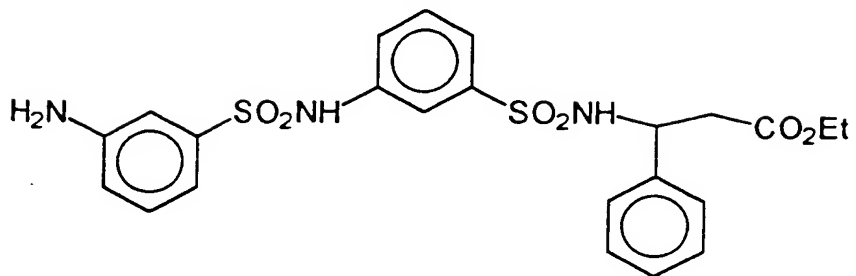


10 By utilizing the same procedure as described in Example E, the product from Example F was coupled with 3-nitrobenzene sulfonyl chloride to afford the above compound.

NMR data was consistent with the proposed structure.

Example BB

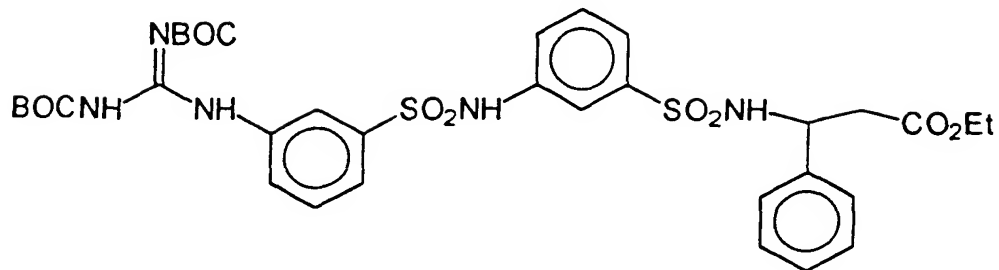
5



10 The product from Example AA was hydrogenated and purified in the same manner as described in Example F.

NMR data was consistent with the proposed structure.

Example CC

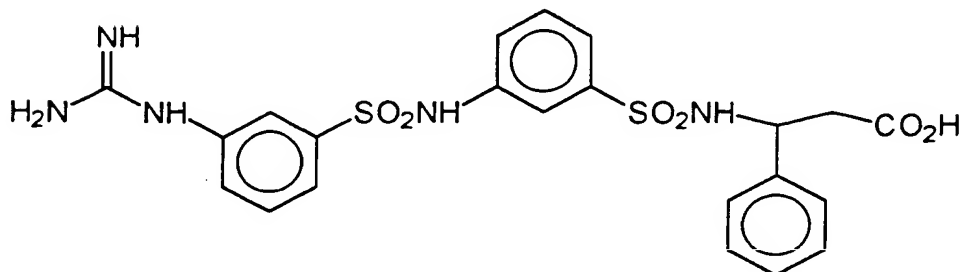


To a solution of the product from Example BB, 1.2 equivalents of bis-t-butoxycarbonyl thiourea and 2.2 equivalents of triethylamine in DMF at 0° under nitrogen was added 1.2 equivalents mercuric chloride in one portion. The reaction was stirred for 30 minutes at 0° and then 30 minutes at room temperature. The reaction was quenched with ethyl acetate, stirred for 30 minutes, and then filtered and concentrated. The crude product was purified on a silica gel column eluting with 25% ethyl acetate - 75% hexane to afford the product.

NMR data was consistent with the proposed structure.

Example 9

$\beta$ -[[[3-[[[3-[(aminoiminomethyl)amino]phenyl]-sulfonyl]amino]phenyl]sulfonyl]amino]phenyl-propanoic acid, trifluoroacetate salt



5

The product from Example CC was treated and purified in the same fashion as described in Example 1.

10 NMR data was consistent with the proposed structure.

Analysis Calc'd for  $C_{22}H_{23}N_5O_6S_2 \cdot 1.5 CF_3CO_2H$ :

C, 43.61; H, 3.59; N, 10.17; S, 9.31

Found: C, 43.71; H, 3.46; N, 10.34; S, 9.65



The activity of the compounds of the present invention was tested in the following assays. The results of testing in the assays are tabulated in Table 1.

5

#### VITRONECTIN ADHESION ASSAY

##### MATERIALS

Human vitronectin receptor( $\alpha_v\beta_3$ ) was purified from human placenta as previously described [Pytela et al.,  
10 Methods in Enzymology, 144:475-489 (1987)]. Human vitronectin was purified from fresh frozen plasma as previously described [Yatohgo et al., Cell Structure and Function, 13:281-292 (1988)]. Biotinylated human vitronectin was prepared by coupling NHS-biotin from  
15 Pierce Chemical Company (Rockford, IL) to purified vitronectin as previously described [Charo et al., J. Biol. Chem., 266(3):1415-1421 (1991)]. Assay buffer, OPD substrate tablets, and RIA grade BSA were obtained from Sigma (St. Louis, MO). Anti-biotin  
20 antibody was obtained from Calbiochem (La Jolla, CA). Linbro microtiter plates were obtained from Flow Labs (McLean, VA). ADP reagent was obtained from Sigma (St. Louis, MO).

##### 25 METHODS

##### Solid Phase Receptor Assays

This assay was essentially the same as previously reported [Niiya et al., Blood, 70:475-483 (1987)]. The  
30 purified human vitronectin receptor ( $\alpha_v\beta_3$ ) was diluted from stock solutions to 1.0  $\mu\text{g/mL}$  in Tris-buffered saline containing 1.0 mM  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , and  $\text{Mn}^{++}$ , pH 7.4 (TBS $^{+++}$ ). The diluted receptor was immediately transferred to Linbro microtiter plates at 100  $\mu\text{L/well}$   
35 (100 ng receptor/well). The plates were sealed and incubated overnight at 4°C to allow the receptor to bind to the wells. All remaining steps were at room

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temperature. The assay plates were emptied and 200  $\mu$ L of 1% RIA grade BSA in TBS<sup>+++</sup> (TBS<sup>+++</sup>/BSA) were added to block exposed plastic surfaces. Following a 2 hour incubation, the assay plates were washed with TBS<sup>+++</sup> using a 96 well plate washer. Logarithmic serial dilution of the test compound and controls were made starting at a stock concentration of 2 mM and using 2 nM biotinylated vitronectin in TBS<sup>+++</sup>/BSA as the diluent. This premixing of labeled ligand with test (or control) ligand, and subsequent transfer of 50  $\mu$ L aliquots to the assay plate was carried out with a CETUS Propette robot; the final concentration of the labeled ligand was 1 nM and the highest concentration of test compound was  $1.0 \times 10^{-4}$  M. The competition occurred for two hours after which all wells were washed with a plate washer as before. Affinity purified horseradish peroxidase labeled goat anti-biotin antibody was diluted 1:3000 in TBS<sup>+++</sup>/BSA and 125  $\mu$ L were added to each well. After 30 minutes, the plates were washed and incubated with OPD/H<sub>2</sub>O<sub>2</sub> substrate in 100 mM/L Citrate buffer, pH 5.0. The plate was read with a microtiter plate reader at a wavelength of 450 nm and when the maximum-binding control wells reached an absorbance of about 1.0, the final A<sub>450</sub> were recorded for analysis. The data were analyzed using a macro written for use with the EXCEL™ spreadsheet program. The mean, standard deviation, and %CV were determined for duplicate concentrations. The mean A<sub>450</sub> values were normalized to the mean of four maximum-binding controls (no competitor added) (B-MAX). The normalized values were subjected to a four parameter curve fit algorithm [Rodbard et al., Int. Atomic Energy Agency, Vienna, pp 469 (1977)], plotted on a semi-log scale, and the computed concentration corresponding to inhibition of 50% of the maximum binding of biotinylated vitronectin (IC<sub>50</sub>) and corresponding R<sup>2</sup> was reported for those compounds exhibiting greater than 50% inhibition at the

highest concentration tested; otherwise the  $IC_{50}$  is reported as being greater than the highest concentration tested.  $\beta$ -[[2-[[5-[(aminoiminomethyl)amino]-1-oxopentyl]amino]-1-oxoethyl]amino]-3-pyridinepropanoic acid [USSN 08/375,338, Example 1] which is a potent  $\alpha_v\beta_3$  antagonist ( $IC_{50}$  in the range 3-10 nM) was included on each plate as a positive control.

10                    PURIFIED IIb/IIIa RECEPTOR ASSAY

MATERIALS

Human fibrinogen receptor ( $\alpha_{IIb}\beta_3$ ) was purified from outdated platelets. (Pytela, R., Pierschbacher, M.D., Argraves, S., Suzuki, S., and Rouslahti, E. "Arginine-Glycine-Aspartic acid adhesion receptors", Methods in Enzymology 144(1987):475-489.) Human vitronectin was purified from fresh frozen plasma as described in Yatohgo, T., Izumi, M., Kashiwagi, H., and Hayashi, M., "Novel purification of vitronectin from human plasma by heparin affinity chromatography," Cell Structure and Function 13(1988):281-292. Biotinylated human vitronectin was prepared by coupling NHS-biotin from Pierce Chemical Company (Rockford, IL) to purified vitronectin as previously described. (Charo, I.F., Nannizzi, L., Phillips, D.R., Hsu, M.A., Scarborough, R.M., "Inhibition of fibrinogen binding to GP IIb/IIIa by a GP IIIa peptide", J. Biol. Chem. 266(3)(1991): 1415-1421.) Assay buffer, OPD substrate tablets, and RIA grade BSA were obtained from Sigma (St. Louis, MO). Anti-biotin antibody was obtained from Calbiochem (La Jolla, CA). Linbro microtiter plates were obtained from Flow Labs (McLean, VA). ADP reagent was obtained from Sigma (St. Louis, MO).

## METHODS

### Solid Phase Receptor Assays

This assay is essentially the same reported in  
5 Niiya, K., Hodson, E., Bader, R., Byers-Ward, V.  
Koziol, J.A., Plow, E.F. and Ruggeri, Z.M., "Increased  
surface expression of the membrane glycoprotein  
IIb/IIIa complex induced by platelet activation:  
Relationships to the binding of fibrinogen and platelet  
10 aggregation", Blood 70(1987):475-483. The purified  
human fibrinogen receptor ( $\alpha_{IIb}\beta_3$ ) was diluted from  
stock solutions to 1.0  $\mu\text{g/mL}$  in Tris-buffered saline  
containing 1.0 mM  $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , and  $\text{Mn}^{++}$ , pH 7.4 ( $\text{TBS}^{+++}$ ).  
The diluted receptor was immediately transferred to  
15 Linbro microtiter plates at 100  $\mu\text{L/well}$  (100 ng  
receptor/well). The plates were sealed and incubated  
overnight at 4°C to allow the receptor to bind to the  
wells. All remaining steps were at room temperature.  
The assay plates were emptied and 200  $\mu\text{L}$  of 1% RIA  
20 grade BSA in  $\text{TBS}^{+++}$  ( $\text{TBS}^{+++}/\text{BSA}$ ) were added to block  
exposed plastic surfaces. Following a 2 hour  
incubation, the assay plates were washed with  $\text{TBS}^{+++}$   
using a 96 well plate washer. Logarithmic serial  
dilution of the test compound and controls were made  
25 starting at a stock concentration of 2 mM and using 2  
nM biotinylated vitronectin in  $\text{TBS}^{+++}/\text{BSA}$  as the  
diluent. This premixing of labeled ligand with test  
(or control) ligand, and subsequent transfer of 50  $\mu\text{L}$   
30 aliquots to the assay plate was carried out with a  
CETUS Propette robot; the final concentration of the  
labeled ligand was 1 nM and the highest concentration  
of test compound was  $1.0 \times 10^{-4}$  M. The competition  
occurred for two hours after which all wells were  
washed with a plate washer as before. Affinity  
35 purified horseradish peroxidase labeled goat anti-  
biotin antibody was diluted 1:3000 in  $\text{TBS}^{+++}/\text{BSA}$  and 125  
 $\mu\text{L}$  were added to each well. After 30 minutes, the

plates were washed and incubated with ODD/H<sub>2</sub>O<sub>2</sub> substrate in 100 mM/L citrate buffer, pH 5.0. The plate was read with a microtiter plate reader at a wavelength of 450 nm and when the maximum-binding control wells reached an absorbance of about 1.0, the final A<sub>450</sub> were recorded for analysis. The data were analyzed using a macro written for use with the EXCEL™ spreadsheet program. The mean, standard deviation, and %CV were determined for duplicate concentrations. The mean A<sub>450</sub> values were normalized to the mean of four maximum-binding controls (no competitor added) (B-MAX). The normalized values were subjected to a four parameter curve fit algorithm, [Robard et al., Int. Atomic Energy Agency, Vienna, pp 469 (1977)], plotted on a semi-log scale, and the computed concentration corresponding to inhibition of 50% of the maximum binding of biotinylated vitronectin (IC<sub>50</sub>) and corresponding R<sup>2</sup> was reported for those compounds exhibiting greater than 50% inhibition at the highest concentration tested; otherwise the IC<sub>50</sub> is reported as being greater than the highest concentration tested.  $\beta$ -[[2-[[5-[(aminoiminomethyl)amino]-1-oxopentyl]amino]-1-oxoethyl]amino]-3-pyridinepropanoic acid [USSN 08/375,338, Example 1] which is a potent  $\alpha_v\beta_3$  antagonist (IC<sub>50</sub> in the range 3-10 nM) was included on each plate as a positive control.

#### Human Platelet Rich Plasma Assays

Healthy aspirin free donors were selected from a pool of volunteers. The harvesting of platelet rich plasma and subsequent ADP induced platelet aggregation assays were performed as described in Zucker, M.B., "Platelet Aggregation Measured by the Photometric Method", Methods in Enzymology 169(1989):117-133. Standard venipuncture techniques using a butterfly allowed the withdrawal of 45 mL of whole blood into a 60 mL syringe containing 5 mL of 3.8% trisodium

citrate. Following thorough mixing in the syringe, the anti-coagulated whole blood was transferred to a 50 mL conical polyethylene tube. The blood was centrifuged at room temperature for 12 minutes at 200 xg to sediment non-platelet cells. Platelet rich plasma was removed to a polyethylene tube and stored at room temperature until used. Platelet poor plasma was obtained from a second centrifugation of the remaining blood at 2000 xg for 15 minutes. Platelet counts are typically 300,000 to 500,000 per microliter. Platelet rich plasma (0.45 mL) was aliquoted into siliconized cuvettes and stirred (1100 rpm) at 37°C for 1 minute prior to adding 50 uL of pre-diluted test compound. After 1 minute of mixing, aggregation was initiated by the addition of 50 uL of 200 uM ADP. Aggregation was recorded for 3 minutes in a Payton dual channel aggregometer (Payton Scientific, Buffalo, NY). The percent inhibition of maximal response (saline control) for a series of test compound dilutions was used to determine a dose response curve. All compounds were tested in duplicate and the concentration of half-maximal inhibition ( $IC_{50}$ ) was calculated graphically from the dose response curve for those compounds which exhibited 50% or greater inhibition at the highest concentration tested; otherwise, the  $IC_{50}$  is reported as being greater than the highest concentration tested.

| Example | AvB3<br>IC50<br>(nM) | IIb/IIIa<br>IC50<br>(nM) | Human PRP<br>( $\mu$ M) |
|---------|----------------------|--------------------------|-------------------------|
| 1       | 1.66                 | 11.3                     | >200 $\mu$ M            |
| 2       | 35.1                 | 353                      |                         |
| 3       | 5.34                 | 11.6                     |                         |
| 4       | 659                  | 987                      |                         |
| 5       | 4.66                 | 24.7                     |                         |
| 6       | 183                  | 3920                     |                         |
| 7       | 15.0                 | 418                      |                         |
| 8       | 11.3                 | 38.2                     |                         |
| 9       | 29.3                 | 93.6                     |                         |